

# The vocalization of the red junglefowl is a signal of body size and individual health

Peipei Hao (郝佩佩)<sup>a</sup>, Kai Zhao (赵凯)<sup>a</sup>, Xi Huang (黄希)<sup>a</sup>, Xiaodong Rao (饶晓东)<sup>b</sup>, Wei Liang (梁伟)<sup>c,\*</sup> and Yanyun Zhang (张雁云)<sup>a,\*</sup>

<sup>a</sup>Ministry of Education Key Laboratory for Biodiversity and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing 100875, China

<sup>b</sup>College of Forestry/Wuzhishan National Long Term Forest Ecosystem Monitoring Research Station, Hainan University, Haikou 570228, China

<sup>c</sup>Ministry of Education Key Laboratory for Ecology of Tropical Islands, College of Life Sciences, Hainan Normal University, Haikou 571158, China

\*Address correspondence to Wei Liang, E-mail: [liangwei@hainnu.edu.cn](mailto:liangwei@hainnu.edu.cn) and Yanyun Zhang, E-mail: [zhangyy@bnu.edu.cn](mailto:zhangyy@bnu.edu.cn)

Handling editor: Zhi-Yun Jia

## Abstract

Bird songs are considered a sexually selected trait that can reflect the individual health of males as well as the vitality of potential mates and their competitors. Vocalization features should, therefore, be related to parasite load and body condition. Here, we performed a thorough acoustic analysis of the display calls of 9-month-old sub-adults and 18-month-old adults of pasture-raised red junglefowls *Gallus gallus*. We investigated whether the calls of pasture-raised red junglefowls can indicate body size and body condition, in addition to the influence of parasitic infection intensity on the expression of sexual traits. We found that frequency-related syllable parameters were significantly positively correlated with wing length in adults, whereas body weight was independent of both the frequency and temporal parameters of calls. In addition, we correlated parasitic load with the expression of sexually selected traits and discovered a positive association between the intensity of parasite infection and wing length in sub-adults, independent of vocal parameters. Overall, the results suggest that the vocalization of red junglefowls may convey reliable body size information, which will facilitate further studies of different vocal parameters in the transmission of bird vocalizations.

**Key words:** blood parasite, male quality, morphology, red junglefowl *Gallus gallus*, vocalization.

Vocalization reflects the size, health, and fighting ability of individuals and plays a key role in sexual selection (Fischer et al. 2004). Empirical research on vertebrate vocal communication suggests that acoustic characteristics (such as call or syllable repetition rates) may provide information about the quality of the vocalist during sexual interactions (Hardouin et al. 2007; Mason and Burns 2015; Favaro et al. 2017). In several amphibians and birds, the fundamental frequency and formant dispersion are important acoustic features associated with body size (Chen et al. 2022; Marcolin et al. 2022). However, rigorous comparisons of body size and vocal frequency in mammals are rare. A study of body size and vocalization frequency in 91 primates and carnivores clearly demonstrated a strong inverse relationship between body size and vocal frequency, filling a long-standing gap in mammalian bioacoustics (Bowling et al. 2017). Additionally, other acoustic features may be used as indicators of body size (Hesler et al. 2012). Body weight in tanagers (Aves: Thraupidae) is correlated with the average note bandwidth, song bandwidth, frequency shift rate, and temporal song characteristics such as note length, pause length, and note rate (Mason and Burns 2015). Male frigate birds, *Fregata minor*, differed significantly in three vocal characteristics—peak frequency, song deviation, and song consistency (Juola and Searcy 2011). A

calling can, therefore, be a reliable indicator of a male's physical health.

In songbirds, information about the quality of the vocalist is more likely to be encoded in the acoustic structure (e.g., fundamental frequency, vocal tract resonance, and loudness) (Zhao et al. 2018). It has been found that in male–female interactions, song repertoire diversity and song rate may convey information on male quality (Hardouin et al. 2007). However, only a few studies have revealed equivalent roles for the acoustic components of songs (Greig et al. 2013; Marcolin et al. 2022). Larger species are generally thought to sing longer and have higher amplitudes, possibly because of their larger syrinx and larynx (Fletcher 2004). Only a few studies have shown that specific acoustic parameters identified by listeners are used to attract mates or repel same-sex competitors. The ability of Asian particolored bat *Vespertilio sinensis* females to compete for food resources may be reflected in their call duration, syllable rate, and repertoire size (Luo et al. 2017). Furthermore, because bird sounds have a plastic component that more directly signals infection, the characteristics of the calls should correlate with the parasite load (Müller et al. 2013).

Parasitic infection may affect song expression (Lopez-Serna et al. 2021). However, it is unclear whether songs convey

Received 13 May 2022; accepted 4 July 2022

© The Author(s) 2022. Published by Oxford University Press on behalf of Editorial Office, Current Zoology.

This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial License (<https://creativecommons.org/licenses/by-nc/4.0/>), which permits non-commercial re-use, distribution, and reproduction in any medium, provided the original work is properly cited. For commercial re-use, please contact [journals.permissions@oup.com](mailto:journals.permissions@oup.com)

information about the parasitism and health of the host (Garamszegi et al. 2005). Numerous studies have shown that parasites adversely affect song output and complexity (Spencer et al. 2005). In barn swallows, *Hirundo rustica*, song duration was significantly negatively correlated with chew louse load, indicating that adult males with greater resistance to chewing lice were more likely to sing longer songs than normal males (Garamszegi et al. 2005). Tick infestations can affect the song performance of male canaries, *Serinus canaria*, by reducing the song consistency (Müller et al. 2013). However, these results may not be consistent when studying different species. A study on Palearctic-African migratory birds found that males with higher song complexity carried fewer infections than males with lower song complexity (Sorensen et al. 2016). Playback experiments in white-crowned sparrows revealed that infection with *Leucocytozoon* changes the song structure by increasing the song consistency (Gilman et al. 2007). The influence of this parasite on the cost of trait expression in different species is a matter of debate (Lopez-Serna et al. 2021).

Parasitic infection additionally affects host morphology (Merrill et al. 2018). Although parasites are commonly found in birds, their impact on the host is highly dependent on specific host-parasite pairing and host conditions (Gagnon et al. 2020). In wintering ducks, evidence revealed that body mass and wing length are negatively associated with infection by *Leucocytozoon* but not by *Plasmodium* or *Haemoproteus* (Fleskes et al. 2017). The intensity of microfilaria and haemosporidians was negatively correlated with the body condition of the white-necked thrush *Turdus albicollis* (De La Torre et al. 2020). Other studies found no correlation between blood parasites and wing length, weight, or body condition (Fratoni et al. 2021; Shurulinkov et al. 2012; Sorensen et al. 2016). Tailored studies are, therefore, required to determine the

effect of blood parasite infections on certain bird populations (Fleskes et al. 2017).

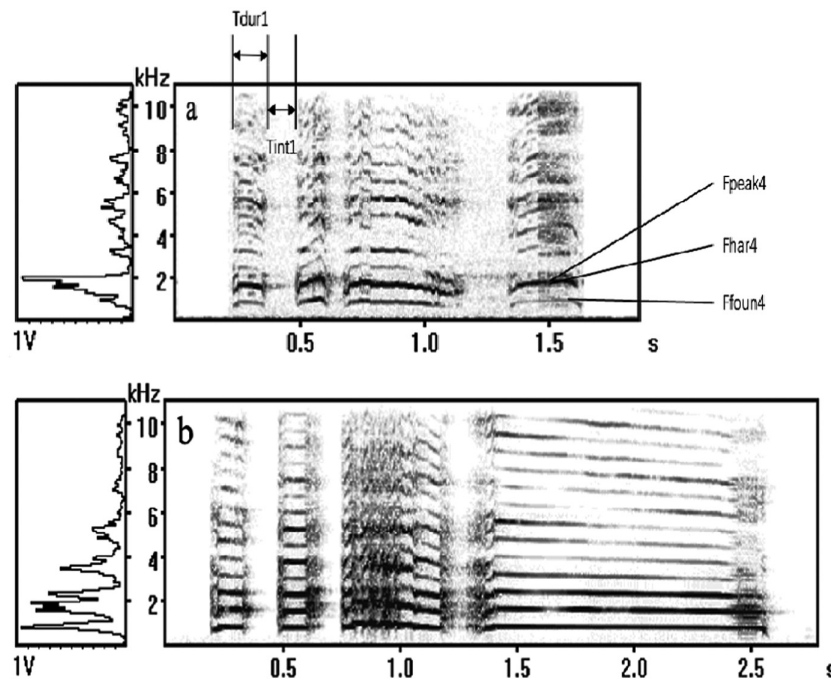
The red junglefowl *Gallus gallus* is a territorial bird, where males produce a series of simple crosses with 4 note sounds to advertise and defend territory (Collias 1987; Tiley et al. 2020). Leonard and Horn (1995) determined that the length of the third syllable was the only crowing feature that correlated with weight in the domestic red junglefowl *Gallus gallus domesticus* (Leonard and Horn 1995). When sexually mature, hens infected with *Ascaridia galli* are lower in weight than non-parasitized birds (Zuk et al. 1998). Compared with other species, the red junglefowl has a clear background, simple calls, and is easy to analyze, making it an ideal choice for studying body conditions and acoustic parameters.

In this study, we conducted a comprehensive analysis of red junglefowl display calls, aiming to address two key questions: 1) Can vocal parameters explain the variance in the body quality of red junglefowl? 2) Does blood parasite infection affect the expression of sexual traits (body quality and vocalization) in red junglefowl? We predicted that red junglefowl vocalizations can serve as a signal of body mass and parasitic infections during the breeding season.

## Materials and Methods

### Study area and species

The fieldwork was conducted on a chicken farm located in Hsinchu town, Dingan' County, Hainan Province (19°37'24"N, 110°12'56"E), covering an area of 0.3 km<sup>2</sup>. The red junglefowls were semi-free range. A total of 20 pasture-raised red junglefowl sub-adults of 9 months (*gallus* sub-adult, hereafter GS) were selected for the experiment, 2 of which lacked parasitic infection data. Additionally, 15 pasture-raised red junglefowl of 18-month adults (*gallus* adult, hereafter GA) were selected, of which 1 lacked audio



**Figure 1.** Spectrograms of the calls of male red junglefowl, showing temporal and frequency measurements: (A) 9-month-old pasture-raised red junglefowl (GS) and (B) 18-month pasture-raised red junglefowl (GA).

recording, morphological data, and parasitic infection data and 2 lacked parasitic infection data. Each individual was placed in a different chicken coop, at least 15 m apart, to ensure that they were not affected by others. Morphological data, blood samples, and song recordings of red junglefowl were collected in April 2021. All the recordings were compiled between 6:00 and 10:00 in the morning and were of sufficient quality to provide clear spectrograms. The recording equipment used was a TASCAM DR-100MKIII recorder (TASCAM, Japan) and Sennheiser MKH416P48 directional microphone (Sennheiser, Germany).

### Morphological measurements

We measured the following 7 morphological characteristics—body mass (kg, with a Pesola spring scale), tail and wing length (cm, with a ruler), head bill length, tarsus length, bill width, and bill depth (mm, with a caliper) (Favaro et al. 2017). All measurements were performed by Peipei Hao, who was systematically trained in bird ringing and measurements. The regression between body weight and wing length was not significant (GS:  $F = 1.09$ ,  $df = 1,18$ , adjusted  $R^2 = 0$ ,  $P = 0.31$ ; GA:  $F = 1.37$ ,  $df = 1,12$ , adjusted  $R^2 = 0.03$ ,  $P = 0.26$ ), and the residual was highly correlated with the body weight (GS:  $F = 296.7$ ,  $df = 1,18$ , adjusted  $R^2 = 0.94$ ,  $P < 0.001$ ; GA:  $F = 105.1$ ,  $df = 1,12$ , adjusted  $R^2 = 0.89$ ,  $P < 0.001$ ). Body weight was considered an indicator of fat reserves and wing length was considered an indicator of body size (Hardouin et al. 2007).

### Parasite quantification

To investigate the impact of parasitic infection on voice and body condition, brachial vein blood was collected from birds and blood DNA was extracted using a TIANamp genomic DNA kit (Beijing Tiangen).

Real-time quantitative PCR (qPCR) was used to quantify the parasite load in each sample using primers 343F–496R, which amplified the parasite's partial mitochondrial rRNA genes (Asghar et al. 2011). All qPCR reactions were performed on a 7500 Real-Time PCR machine using the TB Green Premix reaction kit (Takara Bio Inc., Japan). Each 20- $\mu$ L reaction system contained 2 ng of DNA template and 0.8  $\mu$ L of each primer, and was incubated at 95°C for 30 s, followed by 95°C for 5 s, 52°C for 34 s, and 72°C for 30 s, followed by melting analysis between 60°C and 95°C. All samples were run in duplicates in each reaction. Two nontemplate controls (NTCs) were used to detect false positives, as well as a diluted series of “golden standard samples” (gs). The Ct values for each sample were averaged from amplification curves. The results were accepted only when the fluorescence signal of the NTCs was below the threshold. False-positive tests were performed when Ct values were generated, but the melting peaks were nonspecific. The  $\Delta$ Ct method was used to calculate the relative quantity of positive samples ( $x$ ):  $Q_x = 2^{-(Ct_{gs} - Ct_x)}$  (Huang et al. 2018).

### Sound measurements

We generated spectrograms using the Avisoft-SASLab Pro 4.3.01 audio analysis software (Avisoft Bioacoustics, Germany). The acoustic parameters were measured using the automatic measurement function of the software with

manual assistance using the following settings—fast Fourier transform (FFT), 256 FFT length, Hamming window, 100% frame size, 50% overlap, 86 Hz frequency resolution, and 2.9025 ms temporal resolution. A total of 1,911 recordings were analyzed for GS and 646 recordings for GA. All the recordings were resampled to 11.05 kHz and saved as .wav files. Calls of the red junglefowl have a simple structure, with 4 notes (Fig. 1). Three temporal variables were measured from each spectrogram—the duration of each note (Tdur1, Tdur2, Tdur3, and Tdur4), duration of interval notes (Tint1, Tint2, and Tint3), and call rate. We calculated the natural call rate of red junglefowl by measuring the number of consecutive calls divided by the length in seconds. Additionally, we observed the structure of each note, that is, the frequency of the peak (greatest amplitude) (Fpeak1, Fpeak2, Fpeak3, and Fpeak4), fundamental (Ffoun1, Ffoun2, Ffoun3, and Ffoun4), and first harmonic (Fhar1, Fhar2, Fhar3, and Fhar4) of each note. Due to the syllable's highest energy level in the first harmonic, only the first harmonic values were used. For each individual, we measured 32–197 syllables (mean  $\pm$  SE = 75.21  $\pm$  7.39) and used their mean value for subsequent analysis.

**Table 1.** One-way ANOVA of morphology and vocalization in adults and sub-adults of the red junglefowl.

	ANOVA	
	F	P
Morphology parameters		
Body mass (kg)	67.98	<0.001
Wing length (cm)	31.41	<0.001
Tail length (cm)	13.96	<0.001
Head bill length (mm)	1.74	0.196
Tarsus length (mm)	3.30	0.079
Bill width (mm)	13.20	<0.001
Bill depth (mm)	3.40	0.074
Temporal parameters (s)		
Tdur1	3.30	0.079
Tdur2	2.07	0.160
Tdur3	1.08	0.306
Tdur4	0.02	0.888
Tint1	0.00	0.973
Tint2	2.80	0.104
Tint3	0.12	0.729
Call rate	3.00	0.093
Frequency parameters (Hz)		
Fpeak1	3.22	0.082
Fpeak2	0.22	0.640
Fpeak3	0.33	0.569
Fpeak4	0.84	0.367
Ffoun1	0.06	0.814
Ffoun2	2.10	0.157
Ffoun3	0.60	0.445
Ffoun4	1.71	0.200
Fhar1	0.56	0.461
Fhar2	0.45	0.507
Fhar3	0.23	0.636
Fhar4	0.07	0.796

## Statistical analysis

We performed multivariate and 1-way ANOVA for morphological data and vocal parameters in the adults and subadults, respectively. We then reduced the dimensionality of the syllable's temporal and frequency parameters using principal component analysis (PCA). The frequency parameters of the GA and GS extracted 2 principal components (PC1 and PC2), and the temporal parameter extracted three principal components for subsequent data analysis (PC1, PC2, and PC3). To determine whether acoustic parameters of the red junglefowl could affect body size (wing length) and quality (weight), we used principal component scores as predictor variables and mass and wing length as response variables in multiple linear regression analyses. To determine whether parasite load affected the morphology and acoustics of red junglefowl, we examined the correlation between log parasite infection and morphological parameters using simple linear regression. Subsequently, in the multiple linear regression analysis, we used the syllabic principal component score as a predictor variable and log parasitic infection as the response variable in the multiple linear regression analysis (Zhao et al. 2018).

R Studio was used for all statistical analyses (Version 1.4.1717). The data are presented as mean  $\pm$  SE, and all tests were considered significant at  $P < 0.05$ .

## Results

There were significant differences in morphology between adults and sub-adults of the red junglefowl (Manova:  $F = 26.88$ ,  $df = 7,27$ ,  $P < 0.001$ ,  $N = 35$ ; see Table 1 for univariate ANOVAs) but no differences in acoustic parameters (Manova:  $F = 1.73$ ,  $df = 20,13$ ,  $P = 0.16$ ,  $N = 34$ ; see Table 1 for univariate ANOVAs).

PC1 and PC2 of the GA frequency parameter reflected 82% of the variance, where PC1 included Fpeak2, Fpeak3, Fpeak4, Ffoun1, Ffoun3, Fhar2, and Fhar4, and PC2 included Fpeak1, Ffoun2, Ffoun4, Fhar1, and Fhar3. PC1, PC2, and PC3 of the GA temporal parameters reflected 78% variance, where PC1 included Tdur2, Tint2, and call rate, PC2 included Tdur4 and Tint1, and PC3 included Tdur1 and Tint3. PC1 and PC2 of the GS frequency parameter explained 74% of the variance, among which Ffoun1, Ffoun2, Ffoun3, Ffoun4, Fhar1, Fhar2, Fhar3, Fhar4, Fpeak2, Fpeak3, and Fpeak4 were strongly correlated with PC1, whereas PC2 only represented Fpeak1. PC1, PC2, and PC3 of the GS time parameter explained 72% of the variance. Here, PC1 reflected Tdur3, Tdur4, Tint2, Tint3, and call rate; PC2 reflected Tdur1 and Tint1; and PC3 reflected Tdur2 (Table 2).

## Physical correlates of acoustic parameters

Multiple regression models demonstrated that acoustic parameters were not well predictive of the body weight of

**Table 2.** Results of the PCA for acoustic variables of syllables (see text for abbreviations).

	GA			GS		
	PC1	PC2	PC3	PC1	PC2	PC3
Frequency parameters (Hz)						
Fpeak1	0.58	0.62		0.22	0.87	
Fpeak2	0.91	0.09		0.80	0.26	
Fpeak3	0.90	0.23		0.59	0.58	
Fpeak4	0.93	0.14		0.68	0.46	
Ffoun1	0.73	-0.26		0.89	-0.18	
Ffoun2	0.56	-0.81		0.92	-0.19	
Ffoun3	0.86	-0.17		0.89	-0.04	
Ffoun4	0.47	-0.88		0.86	-0.30	
Fhar1	0.20	0.93		0.76	0.26	
Fhar2	0.74	0.05		0.81	-0.27	
Fhar3	0.19	0.96		0.82	-0.20	
Fhar4	0.91	0.00		0.74	-0.34	
Eigenvalue	6.09	3.76		7.12	1.82	
Percentage of variance	0.51	0.31		0.59	0.15	
Temporal parameters (s):						
Tdur1	-0.57	-0.52	-0.58	-0.30	-0.79	0.33
Tdur2	0.85	-0.04	-0.16	-0.31	0.48	0.75
Tdur3	0.85	-0.20	-0.22	-0.76	-0.11	-0.35
Tdur4	-0.59	0.73	-0.05	-0.82	0.24	-0.13
Tint1	0.57	0.75	0.08	0.40	0.58	-0.21
Tint2	0.79	-0.26	0.40	0.74	-0.25	-0.37
Tint3	-0.40	-0.36	0.79	0.81	0.25	0.18
Call rate	0.35	-0.07	-0.23	0.53	-0.40	0.32
Eigenvalue	3.34	1.62	1.27	3.09	1.54	1.13
Percentage of variance	0.42	0.20	0.16	0.39	0.19	0.14



GA (Frequency:  $F_{2,9} = 0.32$ , adjusted  $R^2 = -0.14$ ,  $P = 0.73$ ; Temporal:  $F_{3,4} = 0.21$ , adjusted  $R^2 = -0.51$ ,  $P = 0.88$ ). A substantial positive association was found between frequency parameters and wing length in GA ( $F_{2,9} = 5.21$ , adjusted  $R^2 = 0.43$ ,  $P = 0.03$ ), whereas there was no correlation observed between wing length and temporal parameters ( $F_{3,4} = 0.76$ , adjusted  $R^2 = -0.11$ ,  $P = 0.57$ ). For GS, it was found that the acoustic parameters were poor predictors of body weight (Frequency:  $F_{2,9} = 0.29$ , adjusted  $R^2 = -0.15$ ,  $P = 0.75$ ; Temporal:  $F_{3,4} = 1.43$ , adjusted  $R^2 = 0.16$ ,  $P = 0.36$ ) and wing length (Frequency:  $F_{2,9} = 0.29$ , adjusted  $R^2 = -0.15$ ,  $P = 0.76$ ; Temporal:  $F_{3,4} = 1.62$ , adjusted  $R^2 = 0.21$ ,  $P = 0.32$ ) (Table 3).

**Relationship between parasitic infection and sexual trait expression**

Simple linear regression and multiple regression analyses demonstrated that the parasitic infection intensity of GA was independent of morphological and acoustic parameters ( $P > 0.05$ ). For GS, we found that parasite infection intensity was only significantly positively correlated with wing length ( $t = -2.45$ ,  $P = 0.03 < 0.05$ ,  $R^2 = 0.23$ ), independent of other morphological parameters ( $P > 0.05$ ). Additionally, there was no association between parasite infection and acoustic parameters

( $F_{\text{Frequency}} = 1.69$ ,  $df = 2,7$ , adjusted  $R^2 = 0.13$ ,  $P = 0.25$ ;  $F_{\text{Temporal}} = 0.94$ ,  $df = 3,3$ , adjusted  $R^2 = -0.03$ ,  $P = 0.52$ ) (Table 4).

**Discussion**

This study revealed the acoustic cues that are correlated to body size. Almost all previous studies have examined avian malaria using binary infection status, but very few have assessed the intensity of infection in plasma (Lopez-Serna et al. 2021). We used qPCR to quantify parasitic loads, which helped to better understand the consequences of parasitic infections on sexual selection characteristics. We found a significant positive correlation between frequency parameters and wing length in GA, and we also found that the blood parasite load was substantially linked to body size in GS, independent of acoustic parameters. It may, therefore, be inferred that the red junglefowl vocalizations reliably convey information about the caller’s body size.

Animal vocalizations are used for mating and defense, and a growing body of research suggests that their size or mass may alter their vocal organs and thus alter the acoustic characteristics of their vocal output (Favaro et al. 2017). Male little penguins, *Edyptula minor*, with larger bone sizes vocalized at lower dominant frequencies during breeding, and females

**Table 3.** Multiple regression of acoustic parameters with body weight and wing length

	Response	Predictor		Estimate	SE	t	P
GA	Body weight	Frequency-	Intercept	2.04	0.19		
			PC1	0.17	0.27	0.63	0.54
			PC2	0.09	0.12	0.69	0.51
		Temporal-	Intercept	2.10	0.10		
			PC1	0.08	0.15	0.52	0.63
			PC2	-0.10	0.20	-0.51	0.64
	Wing length	Frequency-	Intercept	24.92	0.39		
			PC1	0.68	0.54	1.25	0.24
			PC2	0.81	0.25	3.23	<b>0.01</b>
		Temporal-	Intercept	25.47	0.28		
			PC1	-0.33	0.43	-0.77	0.49
			PC2	-0.60	0.59	-1.02	0.37
			PC3	-0.54	0.66	-0.82	0.46
GS	Body weight	Frequency-	Intercept	1.95	0.38		
			PC1	-0.34	0.48	-0.71	0.50
			PC2	-0.17	0.23	-0.75	0.48
		Temporal-	Intercept	1.66	0.06		
			PC1	0.04	0.09	0.43	0.69
			PC2	-0.09	0.13	-0.66	0.55
	Wing length	Frequency-	Intercept	22.54	1.77		
			PC1	1.70	2.25	0.76	0.47
			PC2	0.66	1.08	0.62	0.55
		Temporal-	Intercept	23.62	0.23		
			PC1	-0.44	0.37	-1.19	0.30
			PC2	0.73	0.52	1.41	0.23
			PC3	0.74	0.61	1.21	0.29

Note: Significant correlations are shown in bold ( $P < 0.05$ ).

**Table 4.** Simple linear regression of parasitic infection intensity with morphological parameters and multiple regression with acoustic parameters.

		Parasite Infection intensity (GA)					Parasite Infection intensity (GS)				
		Estimate	SE	<i>t</i> Value	<i>P</i>	R <sup>2</sup>	Estimate	SE	<i>t</i> Value	<i>P</i>	R <sup>2</sup>
Morphological parameters:											
Weight (kg)		-1.82	1.59	-1.14	0.28	0.03	-1.47	1.10	-1.34	0.20	0.04
Wing length (mm)		-0.56	0.61	-0.91	0.39	-0.02	-0.37	0.15	-2.45	<b>0.03</b>	0.23
Tail length (mm)		-0.03	0.06	-0.47	0.65	-0.08	0.02	0.05	0.51	0.61	-0.05
Tarsus length (mm)		-0.04	0.08	-0.50	0.63	-0.07	-0.04	0.04	-0.81	0.43	-0.02
Bill depth (mm)		-0.27	0.25	-1.07	0.31	0.01	-0.18	0.22	-0.80	0.44	-0.02
Bill width (mm)		-0.05	0.27	-0.18	0.87	-0.10	0.20	0.23	0.87	0.40	-0.01
Head bill length (mm)		-0.02	0.09	-0.19	0.85	-0.10	-0.02	0.05	-0.40	0.70	-0.05
Scores of the principal components:											
Frequency	Intercept	-0.31	0.96	-0.32	0.76	-0.26	0.25	1.73	0.14	0.89	0.13
	PC1	-0.25	1.38	-0.18	0.86		-0.48	2.16	-0.22	0.83	
	PC2	-0.21	0.62	-0.34	0.74		-0.95	0.67	-1.42	0.20	
Temporal	Intercept	-0.54	0.45	-1.20	0.32	-0.12	-0.32	0.23	-1.35	0.27	-0.03
	PC1	0.32	0.67	0.47	0.67		0.55	0.34	1.60	0.21	
	PC2	1.43	1.14	1.25	0.30		0.23	0.67	0.34	0.76	
	PC3	-0.76	0.99	-0.77	0.50		-0.40	0.59	-0.67	0.55	

Note: Significant correlations are shown in bold ( $P < 0.05$ ).

were more attracted to these larger individuals (Miyazaki and Waas 2003). Garcia et al. (2014) found that the maximum frequency and emphasized frequency was negatively correlated with body weight in three neotropical cardinals, with heavier species generally having longer notes, lower frequencies, and longer intervals (Garcia et al. 2014). Here, we reported that PC2<sub>Frequency</sub> (Fpeak1, Ffoun2, Ffoun4, Fhar1, and Fhar3) can better predict GA body size, where the frequency was relatively higher in larger individuals, probably because of their increased proclivity for mating or territorial defense competition. However, no significant correlation was found between the weight and acoustic measurements. One possible explanation is that weight only reveals the nutritional status in pasture-raised individuals (i.e., fat storage) (Hardouin et al. 2007). In birds, the vibration rate of syringe membranes ( $f_0$ ) is passively determined by their mass, size, and tension, whereas the nutritional status is thought to determine key factors of their mechanical and functional properties (Favaro et al. 2017). Overall, the results suggest that red junglefowl calls provide reliable cues for individual wing length. However, to date, it has not been determined whether red junglefowl can recognize differences in vocalization between individuals and whether mating is related to the duration and frequency of calls. Playback experiments are needed to test whether recipients use this information.

In songbirds, blood parasites can affect important aspects of host health and sexually selected characteristics such as body size (De La Torre et al. 2020), tail length (Garamszegi et al. 2005), and plumage coloration (Lumpkin et al. 2014; Henschen et al. 2017). This study showed that parasitic infections were mainly related to GS wing length, with smaller individuals having a lower parasitic load. Notably, the study found no association between infection and the other morphological indicators of health. This may be due to the abundant food supply on the farm, which keeps the pasture-raised

red junglefowl in good condition even when infected. Calling, as a sexually selected trait, has been widely reported as an indicator of male health and parasite resistance, indicating that call features should correlate with parasite load (Bischoff et al. 2009; Lopez-Serna et al. 2021). Several surveys have shown that parasite load can affect song consistency—experimental tick infestations altered song performance by reducing the consistency of the vocalizations of male canaries (Müller et al. 2013), infection with *Plasmodium* influenced song structure by reducing song consistency, while infection with *Leucocytozoon* increased song consistency (Gilman et al. 2007). In addition, parasitic infections can also affect call duration and frequency. In male Tawny Owls, parasite infection was significantly negatively correlated with call duration and positively correlated with the frequency of some measured calls (Appleby and Redpath 1997). Some studies have suggested that individuals with a high parasite burden may have lower call rates than those without (Møller 1991; Buchanan et al. 1999; Gilman et al. 2007). Our results demonstrated no significant correlation between the intensity of parasitic infection and acoustic parameters, possibly because of the small sample size (only 15 adults and 20 sub-adults), which may have limited the intensity of testing.

In conclusion, acoustic parameters not only reflect the body size of pasture-raised red junglefowl but also provide information about their health status. This indicates that potential mates and competitors may have the opportunity to learn about their health and, thus, calls may play a role in sexual selection.

## Acknowledgments

The authors are grateful to Xia Canwei for his helpful and valuable suggestions regarding statistical analyses.

## Funding

This work was supported by the National Natural Science Foundation of China (Nos. 32170516 and 31872243 to YZ and 31800320 to XR) and by the Innovation Platform for Academicians of Hainan Province, China.

## Conflict of Interest

The authors declare that there is no competing interest.

## Author Contributions

Y.Z., P.H., and K.Z. conceived and designed the study. X.H. completed quantification of parasitic infection intensity. P.H., K.Z., X.R., and W.L. collected the data. Y.Z. revised the first draft of the manuscript and W.L. edited the manuscript. All authors have approved the final submission.

## Ethics statement

The experiments reported herein complied with the current laws of China. The animal use protocol was reviewed and approved by the Ethics and Animal Welfare Committee, College of Life Sciences, Beijing Normal University (Approval No. CLS-EAW-2021-020).

## References

- Appleby BM, Redpath SM, 1997. Indicators of male quality in the hoots of tawny owls *Strix aluco*. *J Raptor Res* 31:65–70.
- Asghar M, Hasselquist D, Bensch S, 2011. Are chronic avian haemosporean infections costly in wild birds? *J Avian Biol* 42:530–537.
- Bischoff LL, Tschirren B, Richner H, 2009. Long-term effects of early parasite exposure on song duration and singing strategy in great tits. *Behav Ecol* 20:265–270.
- Bowling DL, Garcia M, Dunn JC, Ruprecht R, Stewart A et al., 2017. Body size and vocalization in primates and carnivores. *Sci Rep* 7:41070.
- Buchanan KL, Catchpole CK, Lewis JW, Lodge A, 1999. Song as an indicator of parasitism in the sedge warbler. *Anim Behav* 57:307–314.
- Chen P, Wang J, Miao J, Dong H, Bao J et al., 2022. Female large odorous frogs *Odorrana graminea* prefer males with higher nonlinear vocal components. *Ecol Evol* 12:e8573.
- Collias NE, 1987. The vocal repertoire of the red junglefowl: A spectrographic classification and the code of communication. *Condor* 89:510–524.
- De La Torre GM, Freitas FF, Fratoni RD, Guaraldo AD, Dutra DD et al., 2020. Hemoparasites and their relation to body condition and plumage coloration of the white-necked thrush *Turdus albicollis*. *Ethol Ecol Evol* 32:509–526.
- Favaro L, Gamba M, Gili C, Pessani D, 2017. Acoustic correlates of body size and individual identity in banded penguins. *PLoS ONE* 12:e0170001.
- Fischer J, Kitchen DM, Seyfarth RM, Cheney DL, 2004. Baboon loud calls advertise male quality: Acoustic features and their relation to rank, age, and exhaustion. *Behav Ecol Sociobiol* 56:140–148.
- Fleskes JP, Ramey AM, Reeves AB, Yee JL, 2017. Body mass, wing length, and condition of wintering ducks relative to hematozoa infection. *J Fish Wildlife Manag* 8:89–100.
- Fletcher NH, 2004. A simple frequency-scaling rule for animal communication. *J Acoust Soc Am* 115:2334–2338.
- Fratoni RdO, de la Torre GM, Freitas FJE, Guaraldo AdC, Manica LT, 2021. From unwanted squatters to good tenants: Ectosymbionts and their relationships with body condition of Atlantic forest passeriformes. *Austral Ecol* 46:521–531.
- Gagnon R, Mabika C, Bonenfant C, 2020. Distribution and density of oxpeckers on giraffes in Hwange National Park, Zimbabwe. *Afr J Ecol* 58:172–181.
- Garamszegi LZ, Heylen D, Møller AP, Eens M, de Lope F, 2005. Age-dependent health status and song characteristics in the barn swallow. *Behav Ecol* 16:580–591.
- Garcia NC, Barreira AS, Kopuchian C, Tubaro PL, 2014. Intraspecific and interspecific vocal variation in three neotropical cardinalids (Passeriformes: Fringillidae) and its relationship with body mass. *Emu* 114:129–136.
- Gilman S, Blumstein DT, Foufopoulos J, 2007. The effect of hemosporean infections on white-crowned sparrow singing behavior. *Ethology* 113:437–445.
- Greig EI, Price JJ, Pruett-Jones S, 2013. Song evolution in Maluridae: Influences of natural and sexual selection on acoustic structure. *Emu* 113:270–281.
- Hardouin LA, Reby D, Bavoux C, Burneleau G, Bretagnolle V, 2007. Communication of male quality in owl hoots. *Am Nat* 169:552–562.
- Henschen AE, Whittingham LA, Dunn PO, 2017. The relationship between blood parasites and ornamentation depends on the level of analysis in the common yellowthroat. *J Avian Biol* 48:1263–1272.
- Hesler N, Mundry R, Sacher T, Coppack T, Bairlein F et al., 2012. Song repertoire size correlates with measures of body size in Eurasian blackbirds. *Behaviour* 149:645–665.
- Huang X, Ellis VA, Joensson J, Bensch S, 2018. Generalist haemosporean parasites are better adapted to a subset of host species in a multiple host community. *Mol Ecol* 27:4336–4346.
- Juola FA, Searcy WA, 2011. Vocalizations reveal body condition and are associated with visual display traits in great frigatebirds *Fregata minor*. *Behav Ecol Sociobiol* 65:2297–2303.
- Leonard ML, Horn AG, 1995. Growing in relation to status in roosters. *Anim Behav* 49:1283–1290.
- Lopez-Serna S, Gonzalez-Quevedo C, Fabio Rivera-Gutierrez H, 2021. Beyond illness: Variation in haemosporean load explains differences in vocal performance in a songbird. *Ecol Evol* 11:18552–18561.
- Lumpkin DC, Murphy TG, Tarvin KA, 2014. Blood parasite infection differentially relates to carotenoid-based plumage and bill color in the American goldfinch. *Ecol Evol* 4:3210–3217.
- Luo B, Lu G, Chen K, Guo D, Huang X et al., 2017. Social calls honestly signal female competitive ability in Asian particoloured bats. *Anim Behav* 127:101–108.
- Marcolin F, Cardoso GC, Bento D, Reino L, Santana J, 2022. Body size and sexual selection shaped the evolution of parrot calls. *J Evol Biol* 35:439–450.
- Mason NA, Burns KJ, 2015. The effect of habitat and body size on the evolution of vocal displays in Thraupidae (tanagers), the largest family of songbirds. *Biol J Linn Soc* 114:538–551.
- Merrill L, Levenson JM, England JC, Osborn JM, Hagy HM, 2018. Blood parasite infection linked to condition of spring-migrating lesser scaup *Aythya affinis*. *Can J Zool* 96:1145–1152.
- Miyazaki M, Waas JR, 2003. Correlations between body size, defensive behaviour and reproductive success in male Little Blue Penguins *Eudyptula minor*: Implications for female choice. *Ibis* 145:98–105.
- Møller AP, 1991. Parasite load reduces song output in a passerine bird. *Anim Behav* 41:723–730.
- Müller W, Heylen D, Eens M, Rivera-Gutierrez HF, Groothuis TGG, 2013. An experimental study on the causal relationships between (ecto-) parasites, testosterone and sexual signalling. *Behav Ecol Sociobiol* 67:1791–1798.
- Shurulinkov P, Chakarov N, Daskalova G, 2012. Blood parasites, body condition, and wing length in two subspecies of yellow wagtail *Motacilla flava* during migration. *Parasitol Res* 110:2043–2051.
- Sorensen MC, Asghar M, Bensch S, Fairhurst GD, Jenni-Eiermann S et al., 2016. A rare study from the wintering grounds provides insight into the costs of malaria infection for migratory birds. *J Avian Biol* 47:575–582.

- Spencer KA, Buchanan KL, Leitner S, Goldsmith AR, Catchpole CK, 2005. Parasites affect song complexity and neural development in a songbird. *Proc Roy Soc B-Biol Sci* 272:2037–2043.
- Tiley GP, Pandey A, Kimball RT, Braun EL, Burleigh JG, 2020. Whole genome phylogeny of *Gallus*: Introgression and data-type effects. *Avian Res* 11:7.
- Zhao X, Jiang T, Gu H, Liu H, Sun C et al., 2018. Are aggressive vocalizations the honest signals of body size and quality in female Asian particoloured bats? *Behav Ecol Sociobiol* 72:116.
- Zuk M, Kim T, Robinson SI, Johnsen TS, 1998. Parasites influence social rank and morphology, but not mate choice, in female red junglefowl *Gallus gallus*. *Anim Behav* 56:493–499.