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# Acoustic Factors Influencing Intraspecific Variation in the Emergency Calls of Calves and Alarm Calls of Sika Deer

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## Abstract

Public pastures in Japan are intended for cattle grazing. However, they suffer significant damage due to Sika deer intrusion. Our goal is to control the behavior of cattle and Sika deer that coexist on public pastures by using their respective languages. This technology is available to public pasture managers. For example, it can simplify the task of gathering cattle, which traditionally requires significant time and effort, while also potentially preventing deer from entering from outside. However, the acoustic characteristics of their vocalizations remain poorly understood. This study investigated the effects of acoustic factors on intraspecific variation in the emergency calls of calves and alarm calls of Sika deer. Each vocalization was analyzed using Praat Phonetic Analysis software, and acoustic parameters were quantified using waveform shape and spectrogram analysis. Twenty-nine parameters, including call duration and fundamental frequencies, were compared across intraspecific variations. Individual differences in the emergency calls of calves were examined, while cluster differences in the alarm calls of Sika deer were examined following cluster classification. There were significant differences in the acoustic parameters of emergency and alarm calls. Each voice was discriminated with a 100% correct response rate. The intraspecific variation was 86.7% for calves' emergency calls and 96.0% for Sika deer alarm calls. Multiple regression analysis identified that parameters such as formants 2, 4, and 5, and the degree of voice breaks, affected intraspecific differences in the emergency calls of calves, whereas parameters such as formants 1 and 2, and bandwidths 2 and 3, affected the alarm calls of Sika deer. The respective coefficients of determination, $R^2$ (adjusted), were 0.58 ($P < 0.001$) for calves and 0.79 ($P < 0.001$) for Sika deer. This study suggests that although the acoustic factors affecting intraspecific variation differ between calls with different meanings, formant 2 is common to both.

**Discipline:** Animal Science
**Additional key words:** vocalization

## Introduction

In Japan, cattle graze in public pastures in mountainous areas, but because there are no physical fences around these pastures, Sika deer can easily enter the area. Because Sika deer and cattle are both herbivores, Sika deer intrusion into pasturelands negatively affects cattle productivity. To develop technology that uses the vocalizations of target animals to control their behavior, it is necessary to collect fundamental data on cattle raised in public pastures and on Sika deer that invade these pastures and damage grasslands. Thus far, we have studied and reported on cattle by focusing on the emergency calls and vocalizations of calves during weaning, and on Sika deer by examining their alarm calls and vocalizations in response to danger. Our purpose is to control the behavior of cattle and Sika deer intermingling within vast public pastures separately. Specifically, we aim to easily summon cattle with their vocalizations and to alert Sika deer to their presence, encouraging the deer to escape the pasture. We believe this will enable us to control the behavior of both species within public pastures using vocalizations. Currently, however, our knowledge of the characteristics of cattle and Sika deer vocalizations is fragmentary. For example, regarding calf emergency calls, we reported that there are individual differences in call duration, fundamental frequency, intensity, and formants (Nakamura & Tejima

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2024). These calls can be divided into two segments based on acoustic characteristics, each with a distinct duration. We also reported that emergency calls are divided into two segments based on acoustic characteristics, and that there are differences in call duration, fundamental frequency, intensity, and formants between the segments (Nakamura et al. 2024). However, we could not determine if either of these two segments of calf vocalizations contained the signal information intended to attract the target cattle. Therefore, we next focused our investigation on the acoustic characteristics of the second segment, which involved opening the mouth. When we extracted and analyzed the acoustic factors of the common parts of spectrograms with similar structures from the second segment of each individual's emergency call, we found that the correct response rate to individual differences in emergency calls using discriminant analysis was 71% (Nakamura & Tooyama 2025b). Analysis using this method suggested that the fundamental frequency had the greatest influence on individual differences in calf emergency calls among the acoustic factors. On the other hand, we compared the two clusters of Sika deer alarm calls and found intraspecific variation in acoustic factors, including call duration, fundamental frequency, and formants (Nakamura & Tooyama 2025a). Moreover, because it is extremely difficult to identify individuals among wild animals living in groups, as opposed to domestic animals, hierarchical cluster analysis has proven an effective tool for classifying vocalizations. In addition, our method does more than simply collect animal vocalizations; it captures specific vocalizations in response to certain stimuli for analysis. In other words, the emergency calls of calves and Sika deer we are analyzing are vocalizations produced under specific conditions and are considered to convey the same meaning within each target species. At present, there are very few reports of cattle vocalizations, particularly regarding the effects of acoustic factors on intraspecific variations. However, there are reports of intraspecific differences in cattle vocalizations. Volodin et al. (2017) reported that adult crossbred Brahman × European cattle bulls produced high- and low-frequency tonal calls but that they exhibited individual differences in call duration, fundamental frequency, and other acoustic factors. Yajuvendra et al. (2013) and Gupta et al. (2018) investigated the vocal characteristics of Karan Fries cows when separated from the herd. They reported individual differences in acoustic factors, such as jitter, shimmer, fundamental frequency, intensity, and formants. They also reported that each cow exhibited a unique spectrogram pattern. However, little research has been conducted on intraspecific variation and acoustic factors affecting vocalizations during specific behaviors, such as weaning and estrus. There have been no reports of such differences in Japanese Black cattle. However, there are few reports on intraspecific variations in Sika deer vocalizations. Yen et al. (2013) examined the acoustic characteristics of two vocalizations, moans and howls, in Formosan Sika deer. Their results showed that discriminant analysis could distinguish individual differences in both vocalizations with a probability greater than 60%. Yang et al. (2012) examined Sichuan Sika deer to identify the acoustic characteristics of their alarm calls. They reported that the fundamental frequency of the alarm call decreased with age and was higher in females than males, suggesting intraspecific variation. However, it is difficult to conclude that acoustic factors influencing intraspecific variation have been identified and systematized, even in reports concerning Sika deer vocalizations.

We believe that controlling the behavior of cattle and Sika deer coexisting on public pastures requires using the languages of both target animals. These calls are believed to have the same meaning and were produced under the same experimental conditions. However, the acoustic characteristics of these vocalizations are not fully understood. In this study, we employed a comprehensive analysis of acoustic factors to investigate their influence on intraspecific variation in each vocalization. Our ultimate goal was to control the behavior of cattle and Sika deer on public pastures using their vocalizations. To achieve this, we must examine the acoustics of these two distinctly different vocalizations.

## Materials and methods

### 1. Emergency voice collection

The emergency calls of calves were collected in August and October 2024. This study included six Japanese Black calves (five males and one female) from the Mountainous Grazing Research Station, NARO, Japan. Their ages and body weights were 5 ± 1 months and 154 ± 28 kg, respectively. Calf rearing methods were the same as those used by Nakamura & Tooyama (2025b). All animals were raised in pastures with their mothers until weaning, when they were moved to where their mothers' calls could no longer be heard. After weaning, the animals were herded into groups and housed in feedlots (4 × 27 m). They were fed artificially dried hay ad libitum. The calves had free access to the water tanks. The experiment's calves exhibited no health issues.

Calf vocalizations were recorded within one day of weaning for six hours (0900 to 1500). Data were collected according to the methods described by Nakamura &

Tooyama (2025b). Figure 1 shows the method of collecting the calf vocalizations and the facility overview. None of the calves could see their mothers or hear their vocalizations. Vocalizations were recorded 6 m away from the vocalizing calf. The ambient sound level, measured using a digital sound-level meter (SL-200U; Customs, Tokyo, Japan), was approximately 40 dB. Calves were identified using an ear tagger. All vocalizations were recorded between 40 and 20,000 Hz in the Linear Pulse Code Modulation (LPCM) recording mode using an Integrated Circuit (IC) recorder (ICD-SX1000, Sony, Tokyo, Japan). Calf vocalizations were recorded by identifying each vocalized individual using its ear tag. The tag number of the vocalized animal was immediately recorded in the IC recorder to allow for individual identification. Although all vocalizations were collected throughout the experimental period (6 h/d), only vocalizations whose source was identified were used for the analysis. Additionally, males and females were combined and treated as a single group, as in Nakamura & Tejima (2024). The acoustic parameters of calf vocalizations were basedon the assumption of individual differences.

**2. Alarm voice collection**

The alarm calls of Sika deer were collected between March and June of 2024, and from January to March of 2025. Seven Sika deer (*C. nippon centralis*; three castrated males and four females) from the Suzaka Zoo (Suzaka City, Nagano Prefecture, Japan) were used in this experiment. The participants' average age was ten years at the beginning of the experiment. The castrated males and females were kept separately. The animals were housed in breeding facilities (14.0 × 22.5 m) without a roof. All Sika deer had ad libitum access to dried hay, supplementary feed, and water.

Recordings were made under conditions of low winds and no precipitation, mostly from 1000 to 1400 on closed days of the zoo to avoid background noise from human activity. Figure 2 shows the method of collecting the Sika deer vocalizations and the facility overview. Vocalizations were recorded 5 to 10 m away from the vocalizing deer. The alarm calls of the deer were recorded using our method, which combines dog barking with the manipulation of animal models (Nakamura & Tooyama 2025a). The Sika deer uttered an alarm call when startled by the experimenter's model. The alarm call is a

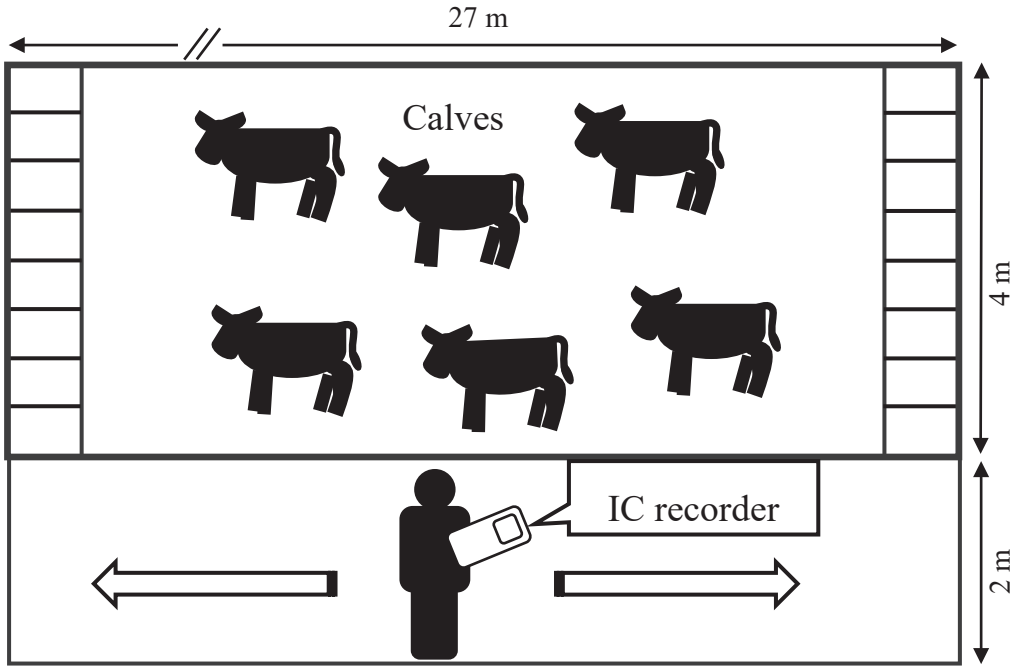



**Fig. 1. Facility overview and method of collecting the calf vocalizations**
The experimenter moved through the passage in step with the herd of calves, checked the individual identification number of the calf that uttered. Calf calls were collected by an IC recorder by individual identification numbers. The distance between the IC recorder and the vocalizing calf was within 6 m. The background noise level was approximately 40 dB. This was created by modifying Figure 1 from Nakamura & Tooyama (2025b).

high-pitched, very short, tension-filled vocalization that involves the entire body. The ambient sound level during measurement with a digital sound level meter (SL-200U) was approximately 40 dB. However, individual identification of each Sika deer vocalization was not possible. All vocalizations were recorded between 40 and 20,000 Hz in the LPCM recording mode using an IC recorder (PCM-A10, Sony, Tokyo, Japan) with a windscreen.

**3. Data analysis**

Recorded vocalizations from all calves and Sika deer were uploaded to a computer at a sampling rate of 44.1 kHz and saved in Waveform Audio File Format (WAV) at a 16-bit amplitude resolution. The number of calf vocalizations was randomized into equal numbers. Vocalizations that could be identified as individual calves' and did not contain noise (e.g., metallic sounds produced by calves hitting fences or bird calls) were extracted and used in the analysis. This was done by visually inspecting the waveform and spectrogram. The study determined the onset and termination of vocalizations in the animals under investigation by performing methods A and B sequentially, as follows. One method involved listening to the vocal sounds and confirming that they belonged to the subject by looking at the sound waveform (A). Another method used formants, which are acoustic factors detected by analysis software alongside vocal sounds (B). This approach leveraged the fact that formants are not detected by analysis software in environments below 40 dB. We extracted the voices under analysis using both methods. In addition, statistical analysis would have been affected by individual differences if the number of vocalizations used for analysis varied across individuals. Therefore, the vocalizations were randomly selected such that each animal had the same number. However, Sika deer were not individually identified.

We used Praat version 6.2.19 (Boersma & Weenink 2022) for the acoustic analyses. An example analysis of calf and Sika deer vocalizations is shown in Figure 3. In this study, the range of the formant displays was defined as a single voice for analysis. This is because the software displays the formant when a sound is produced and

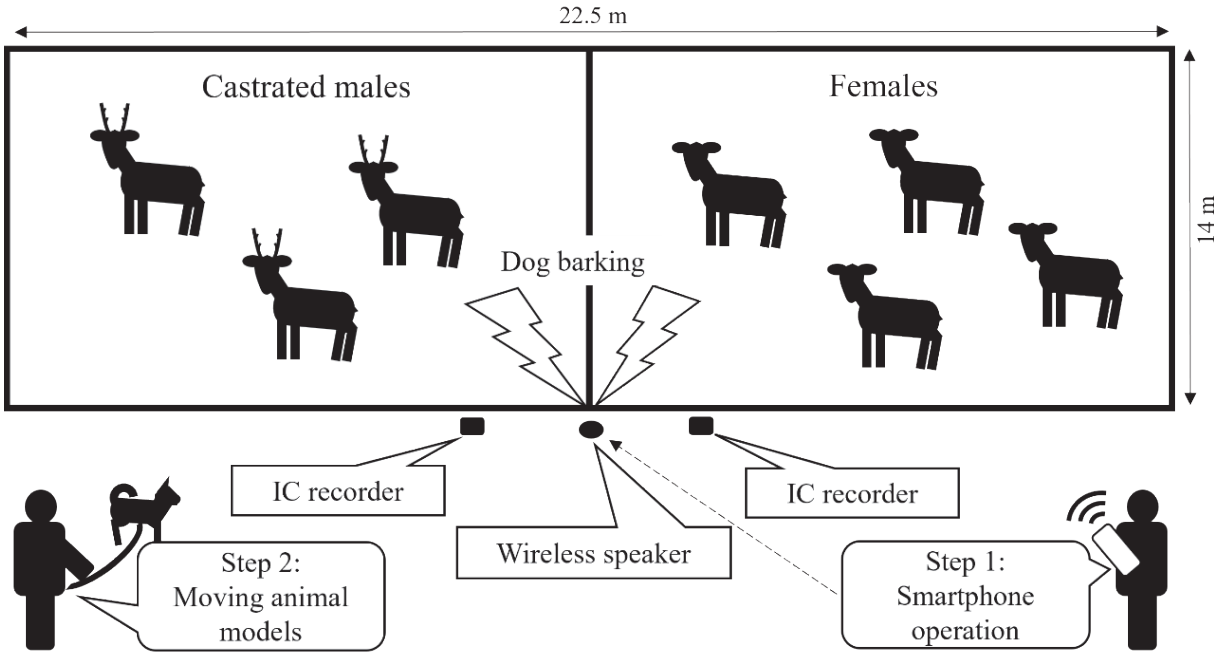



**Fig. 2. Facility overview and method of collecting the Sika deer vocalizations**
Details of steps 1 and 2 are given as follows. The experimenters were not visible to the Sika deer. Step 1: After the equipment was installed, the experimenters used a smartphone to play a pre-recorded sound from a wireless speaker of a dog barking three times. This maneuver put the Sika deer in a state of tension; it stopped acting and watched the source of the vocalization. Step 2: Another experimenter manipulated an animal model and showed it to a Sika deer in a state of tension. By repeating steps 1 and 2, the Sika deer gradually progressed from a state of tension to an alert state and eventually emitted an alarm call, which was recorded by IC recorders. The distance between the IC recorder and the vocalizing Sika deer was 5 to 10 m. The background noise level was approximately 40 dB. This figure was created by modifying Figure 1 from Nakamura & Tooyama (2025a).

immediately hides it when the sound ends, as is the case with the collection of calf vocalizations when the sound level is below 40 dB. In our previous report (Nakamura & Toyama 2025a), we restricted our analysis of vocalizations to the range in which the target animal's formants are represented on the spectrogram. In this study as well, the analysis was limited to the range from the onset to the end of the vocal sounds (Fig. 3). Currently, there are discrepancies in reports on the range of animal vocalizations to be analyzed. Ideally, there should be a unified analytical report on this topic. The conditions for analyzing the calves' voices were as follows: vocalizations were individually visualized as spectrograms in Praat (Fourier method; window length: 0.1 s; time steps: 100; frequency steps: 250; Gaussian window shape; dynamic range: 40 dB; display range: 0 to 5,000 Hz). Vocalizations with high background noise levels were excluded from the analysis. The other settings were as follows: pitch: 50 to 1,500 Hz; intensity: 40 to 120 dB; intensity averaging method: mean energy; formant ceiling: 5,500 Hz; number of formants: 5; formant window length: 0.025; and dynamic range: 30. On the other hand, the conditions for analyzing the Sika deer voice were as follows: their vocalizations were individually visualized on spectrograms in Praat (Fourier method: narrow-band spectrogram; window length: 0.03 s; dynamic range: 55 dB; view range: 0 to 22,000 Hz). Additionally, deer vocalizations recorded in high background noise were excluded from the acoustic analysis. The other settings were as follows (Minami & Kawamichi 1992): pitch: 600 to 3,000 Hz; intensity: 50 to 100 dB; intensity averaging method: mean energy; formant ceiling: 10,000 Hz; number of formants: 5; formant window length: 0.025; and dynamic range: 30.

Abbreviations and definitions for the vocal parameters analyzed in calf and Sika deer vocalizations are shown in Table 1 (see the Praat manual for more detailed information; Boersma & Weenink 2022). The vocalization duration was measured from the waveform. We analyzed the fundamental frequency (Hz), intensity (dB), formants 1 to 5 (Hz), bandwidths 1 to 5 (Hz), number of pulses, number of periods, fraction of locally unvoiced frames (%), number of voice breaks, degree of voice breaks (%), jitter (%), shimmer (%), mean

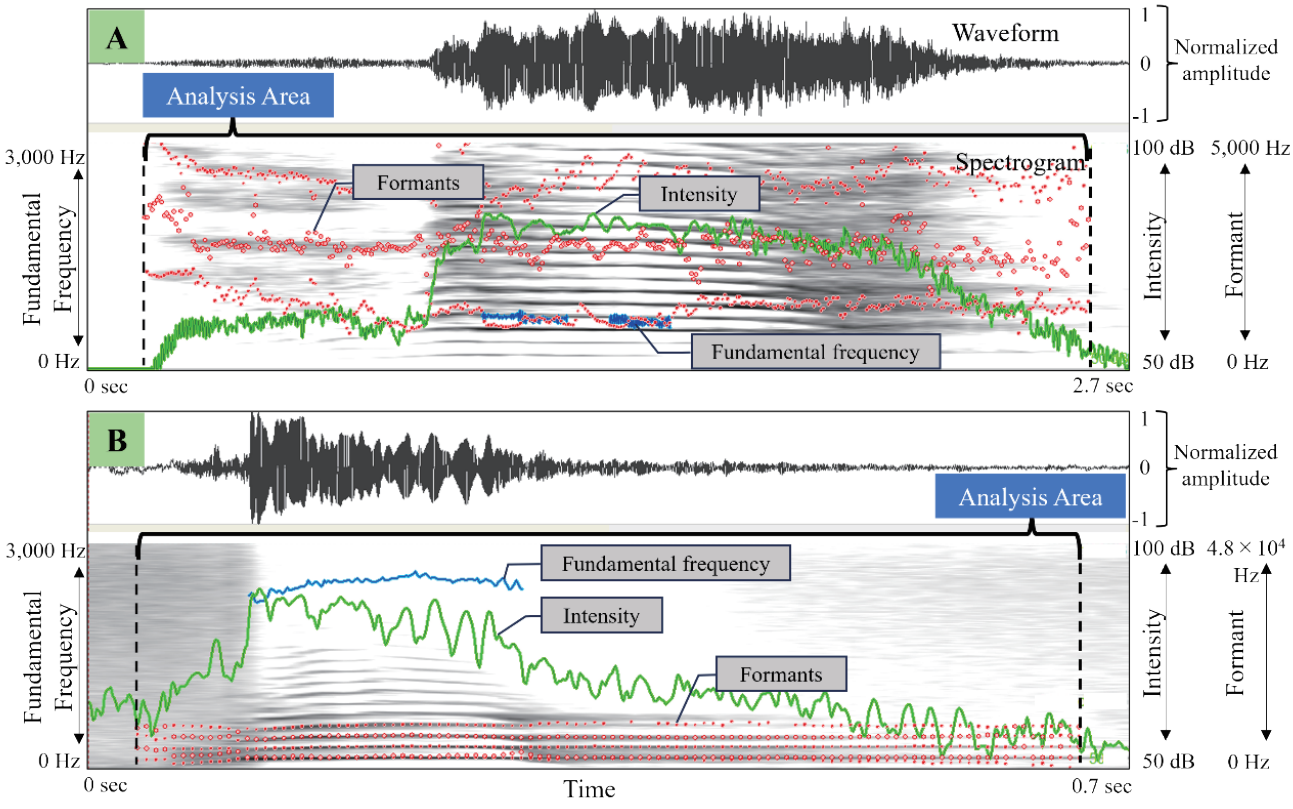



**Fig. 3. Examples of a waveform (top) and spectrograms (bottom)**
A: Vocal parameters in the emergency calls of Japanese Black calves after weaning. B: Vocal parameters in the alarm call of Sika deer. X and Y represent time and frequency, respectively; blue lines represent fundamental frequencies; green lines represent intensity; and red dotted lines represent formant contours.

autocorrelation, mean noise-to-harmonics ratio (%), and mean harmonics-to-noise ratio (dB).

All treatment differences among individual calves were tested using an analysis of variance (ANOVA). The analysis was conducted to detect differences between individuals in each acoustic factor of calf vocalizations. However, in this experiment, it was not possible to identify the individual Sika deer that vocalized. Therefore, we categorized the deer alarm calls based on acoustic parameter similarity. Categorization was performed via hierarchical clustering of all acoustic parameters listed in Table 1. The other settings were as

**Table 1. Abbreviations and definitions of vocal parameters analyzed in calf and Sika deer vocalizations**

| Parameters/Unit of measure | Abbreviation | Definition |
|---|---|---|
| Call duration (s) | Call duration | Total duration of vocalization (from start to end) |
| Fundamental frequency (Hz) | | Subjective auditory sensation and depends on the frequency |
| mean | F (mean) | Mean of the fundamental frequency |
| minimum | F (min) | Minimum of the fundamental frequency |
| maximum | F (max) | Maximum of the fundamental frequency |
| range | F (range) | Difference between minimum and maximum fundamental frequency |
| Intensity (dB) | | The degree of strength of the vocalization |
| mean | I (mean) | Mean of the intensity |
| minimum | I (min) | Minimum of the intensity |
| maximum | I (max) | Maximum of the intensity |
| range | I (range) | Difference between minimum and maximum intensity |
| Formant (Hz) | | Local maximum frequencies in the sound spectrum |
| Formant 1 | F1 | The first harmonic of resonance |
| Formant 2 | F2 | The second harmonic of resonance |
| Formant 3 | F3 | The third harmonic of resonance |
| Formant 4 | F4 | The fourth harmonic of resonance |
| Formant 5 | F5 | The fifth harmonic of resonance |
| Bandwidth (Hz) | | Range of frequencies or transmission speeds used for communication |
| Bandwidth 1 | B1 | The first range of bandwidth |
| Bandwidth 2 | B2 | The second range of bandwidth |
| Bandwidth 3 | B3 | The third range of bandwidth |
| Bandwidth 4 | B4 | The fourth range of bandwidth |
| Bandwidth 5 | B5 | The fifth range of bandwidth |
| Number of pulses | Number of pulses | Pulse is defined as a short transient signal |
| Number of periods | Number of periods | Period is defined as the time to complete a cycle or repetition |
| Fraction of locally unvoiced frames (%) | Fraction of LUF | The percentage of pitch frames analyzed as unvoiced |
| Number of voice breaks | Number of voice breaks | The number of distances between consecutive pulses greater than 1.25 divided by the pitch floor |
| Degree of voice breaks (%) | Degree of voice breaks | Total length of pauses between voices divided by total length of analyzed portion |
| Jitter (%) | Jitter | Frequency variation from cycle to cycle |
| Shimmer (%) | Shimmer | Amplitude variation from cycle to cycle |
| Mean autocorrelation | Mean autocorrelation | Correlation between the values of a signal at different points in time |
| Mean noise-to-harmonics ratio (%) | Noise/Harmonics ratio | Ratio of detectable noise amplitude peaks to harmonic threshold |
| Mean harmonics-to-noise ratio (dB) | Harmonics/Noise ratio | Ratio of detectable harmonic amplitude peaks to noise threshold |

follows: clustering method (Ward's method), measurement method, and squared Euclidean distance. Additionally, the method of division into two clusters was based on Nakamura & Tooyama (2025a), as determined from the dendrogram. Thereafter, all treatment differences were tested using an independent-samples *t*-test among groups to identify discrepancies in acoustic factors among different groups.

All analyzed calf and deer data were grouped using k-nearest neighbor analysis. The call duration, fundamental frequency (mean), and number of voice breaks were extracted from all the acoustic parameters used in the analysis, selected after examining all possible combinations of acoustic factors. On the other hand, Fisher's linear discriminant analysis was performed for each group of data generated by the k-nearest neighbor analysis method to confirm the distinguishability of calf and Sika deer vocalizations. However, "intensity" was excluded from the discriminant analysis because, as mentioned earlier, the distance between the vocalizing individuals and the IC recorder was not constant.

Multiple regression analysis of the acoustic parameters was performed with individual differences for calves and intraspecific differences for Sika deer as the dependent variables. The details of the dependent variables were individual calves (Nos. 1 to 6) for the calves (Tables 2, 3) and individual clusters (Clusters A and B) for the deer (Table 4). First, we assessed multicollinearity and model tolerance for each acoustic parameter and determined that the fundamental frequency (range) in emergency calls of calves and the fundamental frequency (min) in alarm calls of deer did not contribute to explaining the dependent variables in the model. Thus, they were excluded from the calculations because their tolerance was zero. Additionally, VIFs greater than 10 were rejected, even if significant differences were found. This was because other correlations could have confounded the results. Call duration, fundamental frequency (mean), intensity (min, max, range), number of pulses, and number of periods were excluded in calves, and fundamental frequency (mean, max, range), number of pulses, and number of periods were excluded in Sika deer. "Intensity" was also excluded from the multiple regression analysis calculations because, as mentioned earlier, the distance between the vocalizing individuals and the IC recorder was not constant. The forced entry method was used to perform multiple regression analyses after the excluded items were removed as independent variables. Therefore, the coefficient of determination, $R^2$ (adjusted), was calculated excluding items that were excluded from the calculation for zero tolerance and items with a variance inflation factor (VIF) greater than or equal to 10. For exclusion variables, the number of variables assigned to the calculation was temporarily set to 20 for calves and 19 for deer. Pearson's correlation, the standardized coefficient $\beta$, and the VIF were calculated for each dataset. This analysis was conducted to identify the acoustic factors involved in recognizing individual calves and clusters of Sika deer vocalizations.

Statistical analyses were performed using IBM SPSS Statistics Version 21 (IBM, Armonk, NY, USA). This study was conducted in accordance with the NARO regulations for animal experimentation. The Sika deer experiments were approved by R5-W03-NILGS-2.

## Results

A comparison of the acoustic parameters in emergency calls of calves is presented in Tables 2 and 3. Significant differences were found among them by ANOVA, except for the intensity and bandwidths 1, 3, and 5. In detail, the call duration ranged from 1.92 to 2.52 s ($P < 0.001$). The fundamental frequencies (mean, minimum, and maximum) ranged from 100 to 1,149 Hz. However, the fundamental frequency (range) also showed significant differences ($P < 0.001$) across 273 to 1,033 Hz. However, no significant differences were found in the intensity (mean, minimum, maximum, and range). Significant differences ($P < 0.001$) were observed across formants 1 to 5. However, significant differences ($P < 0.01$) were found only for bandwidths 2 and 4. Further, significant differences were found in the number of pulses and periods, fraction of locally unvoiced frames, degree of voice breaks, jitter, shimmer, mean autocorrelation, noise/harmonics ratio, and harmonics/noise ratio at $P < 0.001$, whereas they were found in the number of voice breaks at $P < 0.05$.

A comparison of the acoustic parameters in alarm calls of Sika deer is presented in Table 4. Deer vocalizations were classified into two clusters using cluster analysis. As a result, the acoustic factors that produced differences between deer alarm calls and calf emergency calls were different. Briefly, the call duration in cluster A was significantly longer ($P < 0.05$) than in cluster B. The fundamental frequency (mean, maximum, and range) in cluster A was significantly higher ($P < 0.001$) than in cluster B. The intensity (maximum) in cluster A was significantly higher ($P < 0.001$) than in cluster B. On the other hand, there were significant differences between clusters A and B for all formants and all bandwidths ($P < 0.01$), except for bandwidth 2. Additionally, the shimmer was significantly higher in cluster A ($P < 0.05$), whereas the harmonic/noise ratio

was significantly lower than in cluster B ($P < 0.01$).

The results of the k-nearest neighbor and discriminant analyses are shown in Figure 4. The calves' emergency calls and the Sika deer's alarm calls were classified into distinct groups with a 100% correct class response rate using discriminant analysis. In contrast, the correct response rate for the calves' emergency calls, as determined by individual differences, was 86.7% (Table 3). Similarly, for the alarm call of Sika deer, the correct response rate was 96.0% (Table 4).

Table 5 presents the results of the multiple regression analysis. The acoustic parameters affecting the intraspecific differences in each voice were identified. The respective coefficients of determination, $R^2$ (adjusted), were 0.58 ($P < 0.001$) for calves and 0.79 ($P < 0.001$) for deer. Also, the acoustic factors affecting each species were divided into four types for calves and four for Sika deer. In detail, parameters such as formants 2, 4, and 5, as well as the degree of voice breaks, affected intraspecific differences in the emergency calls of calves. In contrast, parameters such as formants 1 and 2 and bandwidths 2 and 3 affected the alarm calls of Sika deer.

**Table 2. Comparison of acoustic parameters in the emergency calls of weaned Japanese Black calves**

| Parameters | Unit of measure | Calf No. | | | | | | *P*-value (ANOVA) |
|---|---|---|---|---|---|---|---|---|
| | | No.1 | No.2 | No.3 | No.4 | No.5 | No.6 | |
| Number of calls | | 30 | 30 | 30 | 30 | 30 | 30 | |
| sex | | male | male | male | female | male | male | |
| Call duration | s | 1.99 | 1.94 | 1.92 | 2.22 | 2.52 | 2.03 | **0.001** |
| | | ± 0.25 | ± 0.24 | ± 0.25 | ± 0.31 | ± 0.53 | ± 0.26 | |
| F (mean) | Hz | 258 | 264 | 248 | 229 | 234 | 219 | **0.007** |
| | | ± 62 | ± 79 | ± 37 | ± 51 | ± 41 | ± 30 | |
| F (min) | Hz | 106 | 116 | 125 | 128 | 138 | 100 | **0.001** |
| | | ± 36 | ± 14 | ± 28 | ± 28 | ± 20 | ± 14 | |
| F (max) | Hz | 659 | 1,149 | 757 | 400 | 668 | 575 | **0.001** |
| | | ± 349 | ± 409 | ± 447 | ± 131 | ± 373 | ± 300 | |
| F (range) | Hz | 552 | 1,033 | 632 | 273 | 530 | 475 | **0.001** |
| | | ± 340 | ± 411 | ± 453 | ± 139 | ± 364 | ± 299 | |
| I (mean)† | dB | 78 | 76 | 77 | 77 | 77 | 76 | 0.106 |
| | | ± 2 | ± 1 | ± 2 | ± 2 | ± 2 | ± 3 | |
| I (min)† | dB | 55 | 54 | 52 | 54 | 53 | 52 | 0.058 |
| | | ± 5 | ± 4 | ± 4 | ± 5 | ± 5 | ± 5 | |
| I (max)† | dB | 82 | 81 | 82 | 82 | 82 | 81 | 0.052 |
| | | ± 2 | ± 2 | ± 1 | ± 2 | ± 2 | ± 3 | |
| I (range)† | dB | 27 | 27 | 30 | 27 | 28 | 29 | 0.165 |
| | | ± 4 | ± 4 | ± 4 | ± 5 | ± 5 | ± 3 | |
| F1 | Hz | 752 | 1,024 | 998 | 767 | 858 | 776 | **0.001** |
| | | ± 95 | ± 65 | ± 70 | ± 76 | ± 72 | ± 84 | |
| F2 | Hz | 1,556 | 1,766 | 1,735 | 1,531 | 1,740 | 1,609 | **0.001** |
| | | ± 82 | ± 67 | ± 55 | ± 58 | ± 56 | ± 44 | |
| F3 | Hz | 2,595 | 2,692 | 2,679 | 2,520 | 2,749 | 2,517 | **0.001** |
| | | ± 107 | ± 168 | ± 82 | ± 78 | ± 90 | ± 66 | |
| F4 | Hz | 3,774 | 3,882 | 3,840 | 3,693 | 3,733 | 3,548 | **0.001** |
| | | ± 133 | ± 100 | ± 128 | ± 100 | ± 111 | ± 95 | |
| F5 | Hz | 4,751 | 4,601 | 4,637 | 4,660 | 4,617 | 4,398 | **0.001** |
| | | ± 94 | ± 88 | ± 93 | ± 75 | ± 70 | ± 106 | |

Mean ± SD

Details of the parameters are shown in Table 1.

†Because the distance between the vocalizing individual and the IC recorder was not constant, the intensity may have been affected.

## Discussion

In this study, we attempted to identify the acoustic factors that affect intraspecific variation. We collected two vocalizations considered to have the same meaning in the same language for each animal, under identical experimental conditions. First, when the two vocalizations in this study were classified using the k-nearest neighbors method, they were assigned to completely different groups. Based on their discriminant analysis, the classification had a 100% correct response rate (Fig. 4). Next, analysis of variance and discriminant analysis were used to examine the intraspecific differences in the acoustic factors for each vocal sound. A very high probability of correct discrimination was obtained: 86.7% for calf emergency calls and 96.0% for Sika deer alarm calls (Tables 3, 4). Discriminant analysis is commonly used to examine intraspecific differences in vocal sounds. For instance, the following have been reported regarding bovine vocalizations: De la Torrea et al. (2015) studied

**Table 3. Comparison of acoustic parameters in the emergency calls of weaned Japanese Black calves (continued)**

| Parameters | Unit of measure | Calf No. | | | | | | *P*-value (ANOVA) |
|---|---|---|---|---|---|---|---|---|
| | | No.1 | No.2 | No.3 | No.4 | No.5 | No.6 | |
| B1 | Hz | 289 | 385 | 369 | 577 | 372 | 412 | 0.054 |
| | | ± 159 | ± 534 | ± 326 | ± 421 | ± 301 | ± 240 | |
| B2 | Hz | 279 | 604 | 451 | 429 | 254 | 312 | **0.001** |
| | | ± 187 | ± 680 | ± 356 | ± 216 | ± 155 | ± 240 | |
| B3 | Hz | 613 | 870 | 814 | 704 | 628 | 602 | 0.063 |
| | | ± 478 | ± 623 | ± 418 | ± 368 | ± 247 | ± 311 | |
| B4 | Hz | 855 | 866 | 1,264 | 829 | 589 | 922 | **0.004** |
| | | ± 546 | ± 729 | ± 876 | ± 530 | ± 350 | ± 631 | |
| B5 | Hz | 942 | 819 | 809 | 917 | 825 | 1,199 | 0.111 |
| | | ± 524 | ± 529 | ± 418 | ± 445 | ± 411 | ± 1,041 | |
| Number of pulses | | 429 | 381 | 395 | 391 | 508 | 392 | **0.001** |
| | | ± 126 | ± 124 | ± 113 | ± 105 | ± 159 | ± 77 | |
| Number of periods | | 415 | 358 | 382 | 379 | 497 | 381 | **0.001** |
| | | ± 118 | ± 111 | ± 106 | ± 102 | ± 153 | ± 75 | |
| Fraction of LUF | % | 10 | 16 | 10 | 17 | 9 | 6 | **0.001** |
| | | ± 7 | ± 8 | ± 6 | ± 10 | ± 5 | ± 3 | |
| Number of voice breaks | | 2.3 | 1.7 | 2.2 | 2.1 | 1.8 | 1.3 | **0.022** |
| | | ± 1.7 | ± 0.9 | ± 1.5 | ± 1.3 | ± 0.9 | ± 1.0 | |
| Degree of voice breaks | % | 9.1 | 16.2 | 9.9 | 7.8 | 10.0 | 5.7 | **0.001** |
| | | ± 8.4 | ± 8.4 | ± 7.3 | ± 6.7 | ± 7.0 | ± 4.7 | |
| Jitter | % | 3.1 | 1.7 | 2.5 | 3.8 | 1.5 | 2.5 | **0.001** |
| | | ± 1.1 | ± 0.8 | ± 0.6 | ± 2.5 | ± 0.7 | ± 0.7 | |
| Shimmer | % | 15.2 | 12.9 | 16.9 | 15.5 | 10.6 | 12.4 | **0.001** |
| | | ± 5.0 | ± 3.5 | ± 2.4 | ± 4.3 | ± 3.2 | ± 2.5 | |
| Mean autocorrelation | | 0.8 | 0.8 | 0.7 | 0.9 | 0.8 | 0.8 | **0.001** |
| | | ± 0.1 | ± 0.1 | ± 0.1 | ± 0.0 | ± 0.1 | ± 0.0 | |
| Noise/Harmonics ratio | % | 0.3 | 0.2 | 0.4 | 0.1 | 0.2 | 0.3 | **0.001** |
| | | ± 0.1 | ± 0.1 | ± 0.1 | ± 0.1 | ± 0.1 | ± 0.1 | |
| Harmonics/Noise ratio | dB | 9.1 | 9.6 | 5.9 | 12.0 | 10.3 | 9.3 | **0.001** |
| | | ± 3.5 | ± 2.2 | ± 1.4 | ± 2.6 | ± 2.8 | ± 1.8 | |
| The correct response rate, as determined by Fisher's linear discriminant function for discriminating intraspecific variation | | | | | | | | |
| | | | | | **86.7%** | | | |

Mean ± SD
Details of the parameters are shown in Table 1.

the vocal sounds of crossbred beef cattle and their calves during weaning. Using discriminant analysis, they reported individual differences in each vocal sound, with correct response rates ranging from 23% to 53%. Green et al. (2019) investigated vocalizations during estrus and during anticipation of feed in Holstein-Friesian heifers. They reported that discriminant analysis showed that both voices could be distinguished, with response rates of 48% to 68%. Similarly, De la Torrea et al. (2015) and Green et al. (2019) used Praat acoustic analysis software to analyze frequency, formants, jitter, shimmer, and so on. However, reports on the use of discriminant analysis for deer vocal sounds are scarce. Long et al. (1998) reported that discriminant analysis could explain differences in the vocal sounds of red deer, Sika deer, and red × Sika hybrids. Additionally, Yen et al. (2013) demonstrated that discriminant analysis can identify individual differences in the moan and howl vocalizations

**Table 4. Comparison of acoustic parameters between two clusters in the alarm calls of Sika deer**

| Parameters | Unit of measure | Cluster A | Cluster B | *P*-value (*t*-test) |
|---|---|---|---|---|
| Number of calls | | 46 | 54 | |
| Call duration | s | 0.58 ± 0.10 | 0.54 ± 0.10 | **0.048** |
| F (mean) | Hz | 1,905 ± 251 | 1,671 ± 150 | **0.001** |
| F (min) | Hz | 1,247 ± 423 | 1,339 ± 281 | 0.210 |
| F (max) | Hz | 2,225 ± 231 | 1,829 ± 159 | **0.001** |
| F (range) | Hz | 978 ± 407 | 490 ± 309 | **0.001** |
| I (mean)† | dB | 79 ± 1 | 79 ± 1 | 0.411 |
| I (min)† | dB | 54 ± 2 | 53 ± 2 | 0.163 |
| I (max)† | dB | 89 ± 1 | 88 ± 1 | **0.001** |
| I (range)† | dB | 35 ± 2 | 35 ± 2 | 0.635 |
| F1 | Hz | 1,811 ± 162 | 1,655 ± 85 | **0.001** |
| F2 | Hz | 2,732 ± 280 | 3,194 ± 169 | **0.001** |
| F3 | Hz | 4,508 ± 141 | 4,779 ± 286 | **0.001** |
| F4 | Hz | 6,627 ± 157 | 6,867 ± 226 | **0.001** |
| F5 | Hz | 8,715 ± 177 | 8,612 ± 207 | **0.009** |
| B1 | Hz | 395 ± 382 | 193 ± 262 | **0.002** |
| B2 | Hz | 702 ± 911 | 487 ± 679 | 0.179 |
| B3 | Hz | 250 ± 219 | 1,200 ± 865 | **0.001** |
| B4 | Hz | 498 ± 393 | 1,367 ± 1,268 | **0.001** |
| B5 | Hz | 934 ± 533 | 1,923 ± 1,258 | **0.001** |
| Number of pulses | | 337 ± 102 | 308 ± 104 | 0.164 |
| Number of periods | | 332 ± 101 | 304 ± 103 | 0.177 |
| Fraction of LUF | % | 53 ± 12 | 51 ± 10 | 0.385 |
| Number of voice breaks | | 0.6 ± 0.7 | 0.6 ± 0.9 | 0.956 |
| Degree of voice breaks | % | 2.6 ± 4.2 | 3.4 ± 5.5 | 0.407 |
| Jitter | % | 1.9 ± 1.0 | 1.6 ± 0.6 | 0.196 |
| Shimmer | % | 11.8 ± 4.8 | 9.7 ± 3.6 | **0.021** |
| Mean autocorrelation | | 0.9 ± 0.1 | 0.9 ± 0.0 | 0.944 |
| Noise/Harmonics ratio | % | 0.1 ± 0.1 | 0.1 ± 0.0 | 0.190 |
| Harmonics/Noise ratio | dB | 15.4 ± 4.1 | 17.7 ± 3.5 | **0.003** |
| The correct response rate, as determined by Fisher's linear discriminant function for discriminating intraspecific variations | | | **96.0%** | |

Mean ± SD
Details of the parameters are shown in Table 1.
†Because the distance between the vocalizing individual and the IC recorder was not constant, the intensity may have been affected.

of Sika deer during the rut, with a 64% to 74% correct response rate. However, unlike the present study, none of the previous reports conducted an exhaustive analysis of 29 acoustic factors, including bandwidth and other parameters. In other words, the literature and this study's results suggest that increasing the number of acoustic factors analyzed can improve the accuracy of discriminant analyses for examining intraspecific variation in vocal sounds.

Analysis of variance showed individual differences in calf emergency calls, except for intensity (mean, minimum, maximum, and range), and bandwidths 1, 3, and 5 (Tables 2, 3). Jeon et al. (2009) used Holstein dairy calves to characterize the types of vocalizations and their acoustic features. They reported that calf vocalizations were classified as humming, semi-humming, or general, with significant differences ($P < 0.001$) in call duration, fundamental frequency, intensity, and formants 1 to 4. The results showed that general vocalizations had a higher intensity (75 dB) than humming vocalizations (approximately 65 dB). Yeon et al. (2006) compared the acoustic factors of estrus and food-anticipatory calls in native Korean cows and reported significant differences ($P < 0.001$) in intensity and formants 1 to 4. They also found that the intensity of food-anticipating calls (71 dB) was higher than that of the estrus calls (68 dB). In other words, these reports indicated differences in intensity, an acoustic factor, in bovine vocalizations with different meanings. However, the following differences in intensity have been reported in cattle vocalizations that convey the same behavioral meaning: Gupta et al. (2018) examined individual differences in the vocalizations of Karan Fries

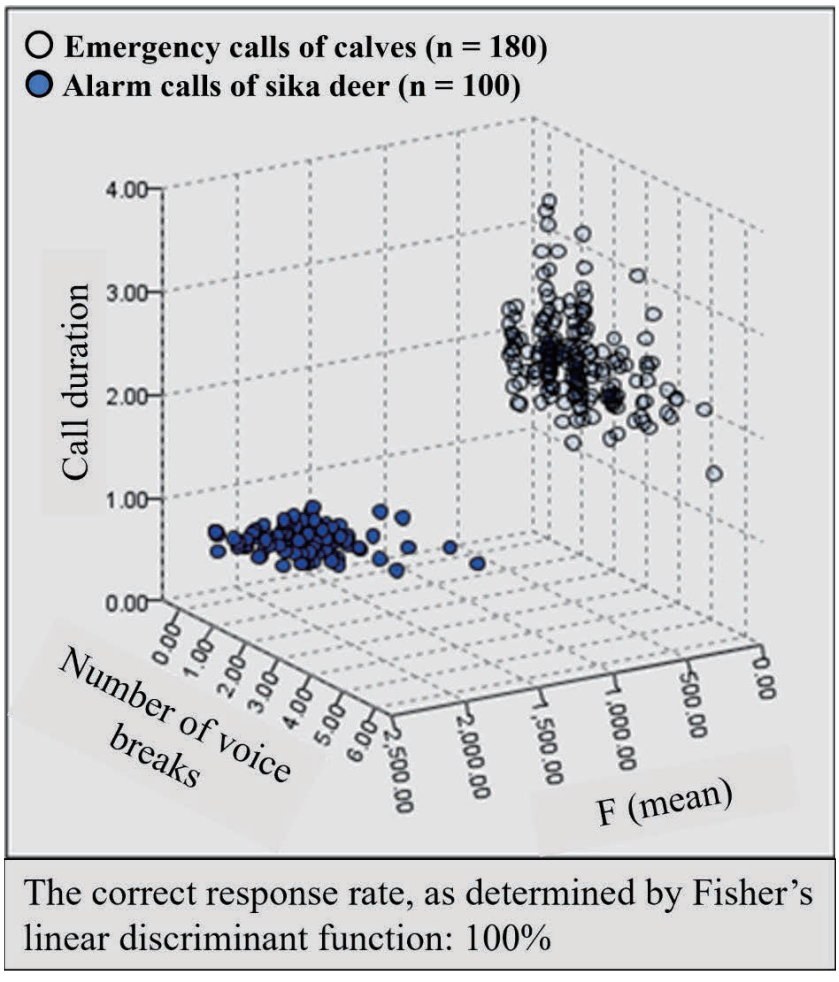



**Fig. 4. Results of the k-nearest neighbor analysis and discriminant analysis using Fisher's linear discriminant function**
White and blue circles indicate emergency calls of calves and alarm calls of deer, respectively.

cows when they were away from the herd. They found significant differences ($P < 0.001$) in various acoustic factors, including intensity, formants 1 to 4, jitter, and shimmer. In a previous report (Nakamura & Tejima 2024), we examined the intraspecific variation in the vocalizations of twenty-four Japanese Black calves during weaning. Our analysis of variance revealed significant differences ($P < 0.001$) in call duration, fundamental frequency, intensity, and formants 1 to 4, indicating individual variation in these vocalizations.

**Table 5. Results of multiple regression analysis**

| | Emergency calls of calves | | Alarm calls of Sika deer | |
|---|---|---|---|---|
| Dependent variable [a] | Individual variations | | Intraspecific variations | |
| Coefficient of determination, $R^2$ (adjusted) [b] | 0.58, $P < 0.001$ | | 0.79, $P < 0.001$ | |
| Results for each coefficient | | | | |
| Parameters | *P*-value | VIF | *P*-value | VIF |
| Call duration | Excluded from calculations | | 0.688 | 2 |
| F (mean) | Excluded from calculations | | Excluded from calculations | |
| F (min) | 0.498 | 1 | Excluded from calculations | |
| F (max) | 0.069 | 2 | Excluded from calculations | |
| F (range) | Excluded from calculations | | Excluded from calculations | |
| I (mean) | Excluded from calculations | | Excluded from calculations | |
| I (min) | Excluded from calculations | | Excluded from calculations | |
| I (max) | Excluded from calculations | | Excluded from calculations | |
| I (range) | Excluded from calculations | | Excluded from calculations | |
| F1 | 0.161 | 3 | **0.001** | 2 |
| F2 | **0.001** | 5 | **0.001** | 3 |
| F3 | 0.067 | 3 | 0.419 | 4 |
| F4 | **0.001** | 3 | 0.169 | 3 |
| F5 | **0.001** | 2 | 0.214 | 2 |
| B1 | 0.161 | 1 | 0.283 | 2 |
| B2 | 0.117 | 1 | **0.006** | 2 |
| B3 | 0.164 | 1 | **0.045** | 2 |
| B4 | 0.328 | 1 | 0.160 | 2 |
| B5 | 0.915 | 1 | 0.136 | 2 |
| Number of pulses | Excluded from calculations | | Excluded from calculations | |
| Number of periods | Excluded from calculations | | Excluded from calculations | |
| Fraction of LUF | 0.259 | 2 | 0.953 | 2 |
| Number of voice breaks | 0.557 | 2 | 0.820 | 3 |
| Degree of voice breaks | **0.037** | 3 | 0.264 | 2 |
| Jitter | 0.397 | 2 | 0.051 | 2 |
| Shimmer | 0.686 | 3 | 0.528 | 6 |
| Mean autocorrelation | 0.473 | 5 | 0.215 | 3 |
| Noise/Harmonics ratio | 0.826 | 8 | 0.421 | 4 |
| Harmonics/Noise ratio | 0.445 | 8 | 0.068 | 8 |

Details of the parameters are shown in Table 1.
VIF: variance inflation factor
[a] The dependent variables were individual calves (Nos. 1 to 6) for the calves (see Tables 2, 3) and individual clusters (Clusters A and B) for the Sika deer (see Table 4), respectively.
[b] The coefficient of determination was calculated excluding items that were excluded from the calculation for zero tolerance and items with a VIF greater than or equal to 10.

However, when we compared the intensity between the 14 male and ten female calves, we found no difference between 76 dB and 75 dB. In contrast, we developed a method that extracts and analyzes common spectral components rather than entire vocal sounds (Nakamura & Tooyama 2025b). This method isolates the obscured portions of the spectrogram at the beginning and end of the vocal sound, and extracts and analyzes only the central portion. Using this method, we analyzed the vocalizations of 13 Japanese Black calves during weaning and found significant differences ($P < 0.001$) in all acoustic factors: call duration, fundamental frequency, intensity, and formants 1 to 5. Additionally, a comparison of the intensities of seven male and six female calves revealed significant differences at 79 dB and 80 dB, respectively. In a previous report (Nakamura & Tejima 2024), we analyzed the full range of calf vocalizations and aligned them based on their distance from the vocalizing calf. However, no individual differences in intensity were observed. The intensity reported by Nakamura & Tejima (2024) was approximately 76 dB, which is similar to the mean intensity in this study, which ranged from 76 dB to 78 dB. However, the lack of individual differences in the mean, minimum, maximum, or range of intensities in this study could not be explained by the available information at this stage. One possible explanation is the distance between the IC recorder and the vocalizing individuals. This was achieved by having the experimenter move in sync with the movements of the calf herd, ensuring that the distance between the vocalizing individuals and the IC recorder remained within 6 m at all times (Fig. 1). In other words, these conditions may have aligned for each individual in this experiment, resulting in no significant difference in intensity, indicating that vocal strength was not observed. Further studies are needed to confirm this hypothesis. Conversely, this study is the first to report the absence of individual differences in vocal sounds during weaning in Japanese Black calves across the bandwidths of 1, 3, and 5. Bandwidth refers to the range of frequencies or transmission speeds used for communication. This study analyzed bandwidths ranging from 1 to 5. There are very few reports on bandwidth measurements using bovine vocalizations. Yajuvendra et al. (2013) examined individual differences in vocal sounds when leaving the herd using Karan Fries crossbred cows. They reported significant differences ($P < 0.001$) in bandwidths 1, 3, and 4, but not in bandwidth 2. This finding is similar to the results of the present study, in which the presence or absence of individual differences depended on the type of bandwidth. In other words, the types of bovine vocalizations differ depending on bandwidth.

Of the 29 acoustic factors examined in this study, most of the acoustic factors of calf emergency calls showed individual differences in the analysis of variance. However, only 16 Sika deer alarm call parameters showed significant differences ($P < 0.05$), as shown in Table 4. In particular, among the fundamental frequency, intensity, formants, and bandwidth, only formants showed significant differences ($P < 0.01$) for all items, whereas the presence of significant differences was mixed for the other items. Minami & Kawamichi (1992) classified Sika deer vocalizations into five major groups based on their lowest and highest fundamental frequencies. However, they did not mention the intraspecific differences in alarm calls. Long et al. (1998) reported that the alarm calls of Sika deer, red deer, and their hybrids could be distinguished within their respective groups. In other words, there are interspecific differences in vocalizations that convey the same meaning. However, there are very few reports on intraspecific variations in alarm calls. For example, Yang et al. (2012) reported that the duration of alarm calls in Sika deer was significantly longer ($P < 0.05$) in males than in females. They also reported that the lowest and highest fundamental frequencies were significantly lower ($P < 0.05$) in males than in females. In a previous report (Nakamura & Tooyama 2025a), we used hierarchical cluster analysis to compare the acoustic factors of two clusters of Sika deer alarm calls. We found significant differences ($P < 0.001$) in call duration, minimum fundamental frequency, and formants 2 to 5 between the two clusters. This indicates that intraspecific variations exist even among vocalizations with the same meaning. Additionally, Yen et al. (2013) discovered individual variations in moan and howl calls using discriminant analysis. These calls are not alarm calls, but rather rutting vocalizations. Thus, the results suggest intraspecific variations in deer vocalizations. However, further studies are required to determine the differences among breeds and types of vocalization. The previous report (Nakamura & Tooyama 2025a) investigated 12 acoustic factors, far fewer than the 29 factors in the present study. The method used to classify the Sika deer alarm calls into two clusters using cluster analysis was the same in both reports. However, the acoustic factors that differed between the two clusters in the previous report and the present study were slightly different. In this study, significant differences ($P < 0.001$) were found in the mean fundamental frequency, maximum intensity, and formant 1 between the two clusters. These differences may be due to the different analysis ranges of the vocalizations of the alarm call. On the other hand, the distance between the IC recorder and the target deer when collecting deer vocalizations in this study was 5-10

meters. Additionally, it was unclear if the target deer consistently vocalized at a constant intensity. Typically, vocalization intensity decreases with increasing distance. Therefore, the significant differences observed in intensity (max) in Table 4 may also be attributed to these factors.

We estimated the acoustic factors affecting intraspecific differences in each species using the acoustic factors for calf emergency calls and Sika deer alarm calls (Tables 2 to 4). Formants 2, 4, 5, and the degree of voice breaks significantly ($P < 0.05$) affected the intraspecific variations in calf emergency calls, whereas formants 1 and 2, as well as bandwidths 2 and 3, significantly ($P < 0.05$) affected the intraspecific variations in deer alarm calls (Table 5). Furthermore, formants 4 and 5 were most likely to affect intraspecific differences in calf emergency calls, as indicated by *P*-values, while formant 2 was most likely to affect intraspecific differences in deer alarm calls. The formant corresponds to the local maximum frequency of the sound spectrum. In this study, the first resonance harmonic was analyzed separately as formant 1. Few studies have attempted to identify the acoustic factors affecting intraspecific variations between the two vocalizations used in this study. In a previous report (Nakamura & Tooyama 2025b), we extracted and analyzed the central portion of the spectrogram of calf emergency calls. By examining the regularity of acoustic parameters using principal component analysis and common logarithms, we found that the fundamental frequency (mean, minimum, and maximum) was the acoustic factor that affected individual differences. However, a direct comparison between the previous report (Nakamura & Tooyama 2025b) and the present study is difficult because, although the animal species and types of vocalizations are the same, the range of vocalizations analyzed differs. This is the first report to identify the acoustic factors affecting intraspecific differences in Sika deer alarm calls. Additionally, formant and bandwidth emerged as common acoustic factors for both vocalizations. The results of this study suggest that various acoustic factors influence intraspecific variation in vocal sounds. In contrast, a method for classifying animal vocalizations using artificial intelligence has been reported in recent years. Briefer et al. (2022) demonstrated the feasibility of establishing animal-welfare-conscious rearing methods by monitoring and classifying pig vocalizations. They also reported that neural networks were more accurate than discriminant function analysis in classifying pig vocal sounds. On the other hand, Liao et al. (2023) proposed TransformerCNN, a new method for recognizing animal sounds and assessing pig health conditions. Using vocalizations made by pigs during four behaviors: calm, feeding, fear, and anxiety, they demonstrated that these behaviors can be classified into different vocal sound categories. They also reported that their method could be used to vocalize animals other than pigs, such as cats, birds, and marine animals. Future use of artificial intelligence rather than traditional analysis methods, such as discriminant analysis, may encourage research into the identification of complex animal vocal sounds.

This study identified issues with experiment reproducibility, particularly the conditions under which vocalizations were collected. For both calves and Sika deer, the distance between the target animal and the IC recorder varied, and the position of the target animal's head changed during vocalization. Furthermore, the recordings of the vocal sounds were made outdoors. This meant that they contained various types of noise. On the other hand, although there were no physical barriers between the IC recorder and the target animals, sound reflections from surrounding structures were also a consideration. These effects may have influenced the acoustic properties of vocalizations, potentially affecting the analysis results. It is necessary to be mindful of these influences when collecting animal vocalizations in the field.

In conclusion, the calf emergency calls and Sika deer alarm calls examined in this study exhibited intraspecific variations despite their shared meaning. Formants were the main factors affecting these differences, although other acoustic parameters also played a role, suggesting that the differences depended on the vocalization being analyzed. On the other hand, the two voices used in this study were acoustically distinct. In other words, we may be able to control the behavior of cattle and Sika deer simultaneously on a public pasture by targeting them with their vocalizations.

## Conflict of interest

The authors declare no potential conflicts of interest.

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