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Paper:

Size Effect on Call Properties of Japanese Tree Frogs Revealed by Audio-Processing Technique

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Sensing the external environment is a core function of robots and autonomous mechanics. This function is useful for monitoring and analyzing the ecosystem for our deeper understanding of the nature and accomplishing the sustainable ecosystem. Here, we investigate calling behavior of male frogs by applying audio-processing technique on multiple audio data. In general, male frogs call from their breeding site, and a female frog approaches one of the males by hearing their calls. First, we conducted an indoor experiment to record spontaneous calling behavior of three male Japanese tree frogs, and then separated their call signals according to independent component analysis. The analysis of separated signals shows that chorus size (i.e., the number of calling frogs) has a positive effect on call number, inter-call intervals, and chorus duration. We speculate that a competition in a large chorus encourages the male frogs to make their call properties more attractive to conspecific females.

Keywords: animal behavior, frog chorus, independent component analysis, microphone array, advertisement call

1. Introduction

Recent development of small and tractable recorders (e.g., video camera and voice recorder) allows us to conduct an efficient monitoring. To monitor ecosystem consisting of multiple animals, however, more dynamical methods are required. Over the decades, robotic research has pursued various technologies for sensing the external world that surrounds the robot [1, 2]. These sensing techniques are indeed useful for monitoring and analyzing the ecosystem for our deeper understanding of the nature and accomplishing the sustainable ecosystem. For example, Kondo and Ura developed an autonomous underwater vehicle with a video camera [3], which is applicable to the monitoring of underwater animals. Tanaka et al. developed an animal monitoring robot with a video camera [4, 5].

While video data is a primary source of information for behavioral observations, audio data is capable of revealing other features of animal behavior. For instance, many species of animals vocalize sounds for various purposes. Bats emit ultrasounds for prey capture as well as obstacle avoidance [6]; male birds sing complex songs to attract conspecific females [7]. The use of a stationary microphone array system attains a detailed analysis of spatio-temporal patterns of their behavior. Fujioka et al. analyzed spatio-temporal dynamics of echolocating bats by calculating time difference of sound arrivals among multiple microphones that were fixed at stationary positions [8]. Suzuki et al. applied a microphone array system to record bird songs in their natural habitat, and discriminated the species of singing birds based on the robot audition system *HARK* [9]. In particular, the bird song analysis attracts interests in the area of robot audition technologies, e.g., special sessions held at international conferences such as ICASSP 2015 and Interspeech 2016. Thus, development of a recording method with a stationary microphone array system is important as a basic technology for monitoring the ecosystem where animals are positioned in a small region, which will be extended to more dynamical method such as a mobile robot equipped

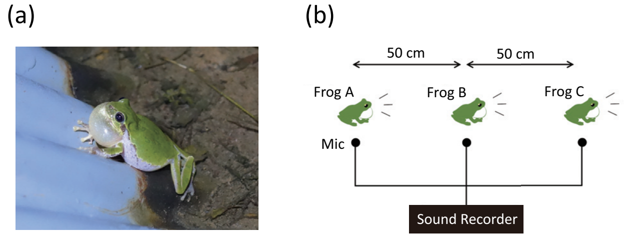

Fig. 1. Recording of frog choruses. (a) Male Japanese tree frog (*Hyla japonica*). (b) Schematic diagram of our indoor experiment. Three male Japanese tree frogs were placed at the interval of 50 cm. Their calling behavior was recorded by three microphones.

with a microphone for revealing the behavior of animals that move around a larger region.

Frogs are abundant nocturnal animals that use sounds during mating process [10–12]. In general, many male frogs simultaneously chorus at a breeding site, and a female frog approaches one of the males by discriminating their calls. Here, our question is posed as follows: what kind of strategy do male frogs have so as to make their calls more attractive in the crowded choruses? To investigate such a calling strategy, it is necessary to discriminate individuals of calling frogs.

We studied calling behavior of male Japanese tree frogs by applying independent component analysis (ICA) on multiple audio data. Japanese tree frogs (*Hyla japonica*) are distributed widely in Japan, from Kagoshima prefecture in the southwest to Hokkaido in the northeast (see **Fig. 1(a)**). Choruses of the male frogs can be observed mainly at paddy fields between April and July [13]. We conducted indoor experiments recording spontaneous calling behavior of the males, and separated their call signals according to ICA [14]. The analysis of the separated signals revealed various types of alternating chorus patterns such as anti-phase synchronization of two frogs and tri-phase synchronization of three frogs. We speculate that the male frogs chorus in such alternating manners to intensely advertise themselves to conspecific females by avoiding call overlaps with each other.

Behavioral experiments using loudspeakers have revealed acoustic preference of female frogs in various species. For instance, female gray tree frogs (*Hyla versicolor*) prefer longer conspecific calls to shorter calls [15], while female African painted reed frogs (*Hyperolius marmoratus*) prefer lower frequency calls to higher frequency calls [16]. Comprehensive studies demonstrate that female frogs tend to prefer call properties deviating from the mean value (e.g., higher call rate and higher loudness) [17]. Thus, a female frog chooses a male frog by comparing their call properties, and then male frogs must compete with each other to attract a female.

In this study, we investigate the relationship between the chorus size and call properties of male Japanese tree frogs. This paper is organized as follows: reviews of our previous study about indoor experiment and independent component analysis (Sections 2.1 and 2.2), evaluation of call properties (Section 2.3), statistical tests (Section 2.4), results (Section 3), discussion (Section 4), and conclusion (Section 5).

Table 1. Weight and body length of male Japanese tree frogs used for our experiment.

Frog ID	A	B	C
Weight	2.4 g	1.9 g	2.7 g
Body length	29.2 mm	29.9 mm	33.6 mm

2. Materials and Methods

2.1. Indoor Experiments

In this study, we use audio data obtained from our previous experiment [14]. In the experiment, three individuals of male Japanese tree frogs were captured at paddy fields of Kyoto University, Japan. Prior to the recording, their weight and body length were measured (see **Table 1**). The frogs were isolated into three small mesh cages, that is, one cage contained one frog. The cages were then placed in an anechoic chamber at the interval of 50 cm (see **Fig. 1(b)**). Their spontaneous calling behavior was recorded overnight with three microphones with the sampling rate of 48 kHz. Temperature and relative humidity of the chamber were 25°C and 52%, respectively. After the recording, the male frogs were released at the same site where they had been captured.

This experiment was conducted on 29th May, 2009, which was cloudy, in accordance with the guidelines approved by the Animal Experimental Committee of Kyoto University. Collection of frogs was permitted by the Experimental Farm of Kyoto University.

2.2. Independent Component Analysis (ICA)

In our previous study [14], we conducted ICA on 3-channel audio data of 4 hours. Here, we review the methods and validity of the analysis as detailed below.

2.2.1. Signal Separation Using ICA

ICA is used for blind source separation (BSS) that is a technique for estimating original source signals using only observed signals that are mixtures of the original signals. In this study, we use the frequency domain ICA (FD-ICA) because its convergence and computational cost are excellent compared with time domain ICA. FD-ICA consists of the following steps (see **Fig. 2**):

1. Conversion from the observed signals to the observed spectra in the short-time Fourier transformation (STFT) domain,

2. Source separation, and solving permutation and scaling problems at each frequency bin,

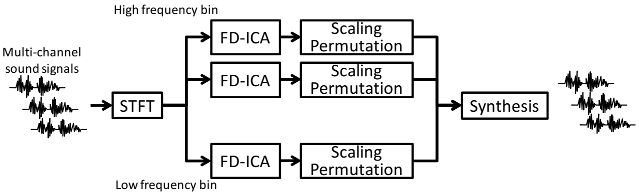

Fig. 2. Overview of FD-ICA.

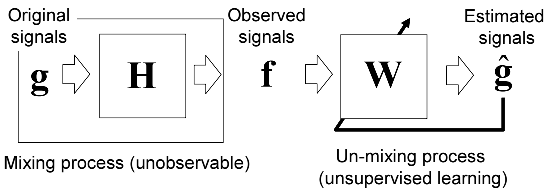

Fig. 3. Flow of ICA processing.

3. Synthesis of signals in the time domain from the estimated spectra.

We explain the algorithm of Step 2 in detail in this section, and omit some techniques such as pre-whitening and initialization of separation matrix [18].

2.2.2. The Model

The flow of ICA processing is outlined in **Fig. 3**. We assume that a zero-mean original source vector, $\vec{g} = [g_1, \ldots, g_L]^T$, consists of L mutually independent complex random variables, $\vec{g} \in \mathbb{C}^L$. They are mixed using a time-invariant linear system that is represented by an $L \times L$ non-singular matrix, $\vec{H} \in \mathbb{C}^{L \times L}$. Let $\vec{f} = [f_1, \ldots, f_L]^T \in \mathbb{C}^L$ be an observed signal vector. The relationship between $\vec{f}$ and $\vec{g}$ is represented as

$$\vec{f} = \vec{H}\vec{g}. \quad \quad (1)$$

ICA estimates original source vector $\vec{g}$ by using only observed vector $\vec{f}$:

$$\hat{\vec{g}} = \vec{W}\vec{f} = \vec{W}\vec{H}\vec{g}, \quad \quad (2)$$

where $\vec{W}$ is an $L \times L$ separation matrix estimated using ICA.

2.2.3. Estimation Algorithm

The higher-order ICA assumes the probabilistic density function (PDF) of $\vec{g}$. ICA estimates $\vec{W}$ by minimizing the Kullback-Leibler divergence (KLD):

$$J(\vec{W}) = \int p(\hat{\vec{g}}) \log \frac{p(\hat{\vec{g}})}{q(\hat{\vec{g}})} d\hat{\vec{g}}, \quad \quad (3)$$

where p is the joint PDF of $\vec{g}$, and q corresponds to the product of the marginal PDF of $\vec{g}$. These parameters are usually estimated using an iterative gradient-based method because of the non-linearity of J.

We use the following iterative equation with nonlinear function based on polar coordinate [19]:

$$\vec{W}^{[j+1]} = \vec{W}^{[j]} - \mu\{\text{off-diag}\langle \vec{I} - \mathbb{E}[\vec{\phi}(\hat{\vec{g}})\hat{\vec{g}}^H]\rangle\}\vec{W}^{[j]} \quad (4)$$

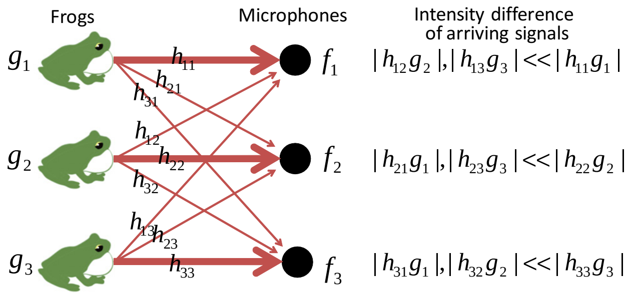

Fig. 4. Mixing process and the power difference in call signals. In our experiment, microphones are set close to respective frogs in an anechoic chamber, making the difference in the power of call signals much clear.

where μ is a step-size parameter, $\mathbb{E}$ is an expectation operator, $\cdot^j$ represents the number of iterations, and $\cdot^H$ represents a conjugate transpose. The operation, off-diag$\langle \vec{X} \rangle$, replaces the diagonal-element of matrix $\vec{X}$ with zero. The $\vec{\phi}$ is a non-linear function vector:

$$\phi(\hat{g}_i) = \tanh(\alpha|\hat{g}_i|)e^{j\theta(\hat{g}_i)}, \quad \quad (5)$$

where α is a gain parameter to control the nonlinearity.

2.2.4. Scaling and Permutation

ICA is ambiguous about the permutation and scaling of each element of the estimated vector, $\hat{\vec{g}}$. These two factors affect the quality of the re-synthesized signals when using ICA in the frequency domain [20].

We use the projection-back method [21] to solve the scaling problem. The estimated signals are then scaled by using elements of the inverse matrix $\hat{\vec{H}} = \vec{W}^{-1}$. The scale ambiguities of the source signal and the estimated separation matrix are canceled out each other.

The permutation of the estimated signals is automatically solved through our estimation process mainly because of 1) the microphone and frog arrangement and 2) the anechoic (non-reverberant) environment for recording. Since we set each microphone close to each frog, the intensity of arriving sound signals of each frog is quite different at each microphone. For example, the power of the signals of the i-th frogs, g_i, is already most dominant than those of others at i-th microphone (**Fig. 4**). This intensity difference becomes clearer in our anechoic environment than in reverberant environment, contributing to align the estimated signals correctly. We speculate that ICA works as the further adjustment of the separation filter in addition to the effect of the intensity difference. It should be noted that we also confirmed the correctness of the permutation by carefully comparing the spectra of the estimated signals with those of the observed signals.

2.2.5. Application of ICA to Frog Choruses

Here, we discuss the validity of the application of ICA to the audio data of frog choruses. In general, the following conditions are required when ICA is applied to sound-source separation [20, 22]:

1. The number of microphones is equal to or larger than that of sound sources.

2. The amplitude histogram of recorded signals, corresponding to the probability density function in the ICA framework, does not follow a Gaussian distribution.

In our experiment, calling behavior of three frogs was recorded by three microphones (see **Fig. 1(b)**), meaning that the first condition holds. To confirm whether the second condition holds, we analyze representative audio data from a single microphone which continue about 15 seconds and include 51 successive calls. Then, the audio data are normalized as its amplitude ranges from -1.0 to $+1.0$ [20]. The histogram of the normalized audio data shows a salient peak (see Supplementary information of [14]). A kurtosis value is then calculated from the histogram [20, 22]. The value is estimated at 13.83, which is much larger than that of Gaussian distribution. Consequently, it is shown that the second condition holds.

For easy and robust detection of call timing, we then calculate the square of separated signals as the power level at each sampling point, and smooth them in every 0.01 second [14].

2.3. Call Properties

To investigate the effect of a chorus size (i.e., the number of calling frogs) on the calling behavior of male Japanese tree frogs, we quantify their call properties from the smoothed call signals. First, we carefully check all the signals, and confirm the occurrence of 58 choruses. Since one of the 58 choruses has a degraded separation quality, we exclude the sample and analyze the rest 57 choruses.

The amplitudes of the signals are then normalized for each chorus. We define the timing of the i-th call vocalized by the n-th frog as $t_{n,i}$. This timing $t_{n,i}$ is estimated by the same method presented in Ref. [14]. From $t_{n,i}$, we estimate the following call properties of the male frogs (see **Fig. 5**):

1. **Call Number:** The number of calls per chorus per frog.

2. **Inter-Call Intervals:** Time intervals between adjacent calls of respective frogs.

3. **Chorus Duration:** Time intervals between the beginning and end of each chorus.

Inter-call intervals are calculated as $\Delta t_{n,i} \equiv t_{n,i+1} - t_{n,i}$ when the following condition is satisfied:

$$0.15 \text{ sec} < \Delta t_{n,i} < 0.75 \text{ sec}. \qquad (6)$$

In our previous study, this range was set at 0.2 sec $<$ $\Delta t_{n,i} < 0.5$ sec as a typical range of the inter-call intervals [23]. However, several exceptions are found through this analysis. Therefore, the range is extended for accomplishing the robust estimation of the intervals.

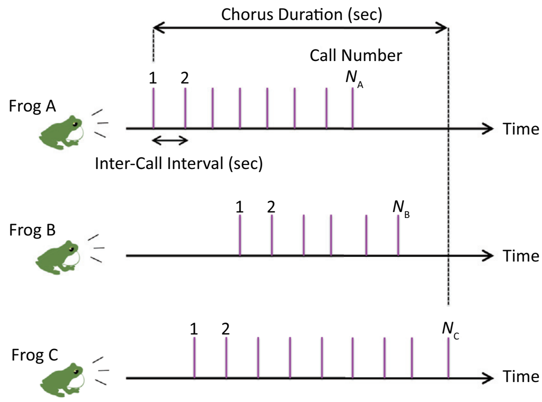

Fig. 5. Definition of call properties. This schematic figure describes a chorus of three frogs. N_A, N_B, and N_C represent call number of respective frogs. Inter-call intervals are calculated as the intervals between adjacent calls.

2.4. Statistical Tests

We analyze the relationship between chorus size and call properties by using two statistical models, i.e., a generalized linear model (GLM) and a generalized linear mixed model (GLMM) [24]. GLM and GLMM are well-known statistical models that are used in various research areas including ethology when analyzing the effect of multiple explanatory variables on a single response variable.

The effect of the chorus size on call number and inter-call intervals is first examined by using GLMM. In the analysis, call number and inter-call intervals are treated as response variables following Poisson distribution and Gamma distribution, respectively. The number of calling frogs is treated as an explanatory variable of a fixed effect. ID of frogs is treated as an explanatory variable of a random effect.

The relationship between the chorus size and chorus duration is then examined by using GLM. Chorus duration is treated as a response variable following Gamma distribution. The number of calling frogs is treated as an explanatory variable of a fixed effect.

All the analyses are performed using R Statistical Software Version 3.1.1. The significance of the fixed effects is estimated by Wald tests [24].

3. Results

Figure 6 shows a representative result of ICA. It is demonstrated that call signals of male Japanese tree frogs are successfully separated; although the components from the other frogs remain in observed signals (see **Fig. 6(a)**), those components disappear in the separated signals (see **Fig. 6(b)**). By analyzing the audio data of 4 hours, we succeeded in detecting 57 choruses (chorus of a single frog: 4, chorus of two frogs: 42, chorus of three frogs: 11).

Figures 7 and **8** show the effect of a chorus size (i.e., the number of calling frogs) on call number and inter-call intervals, respectively. The analysis using GLMM

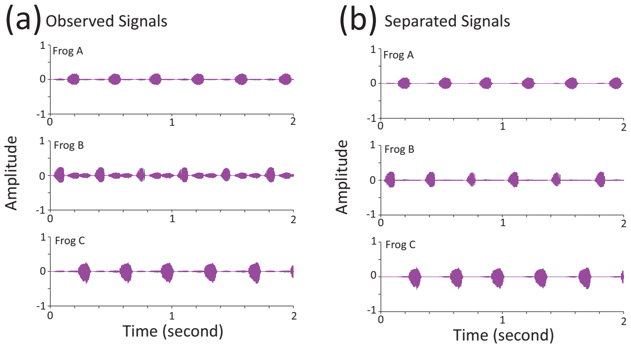

Fig. 6. Result of sound source separation based on ICA. (a) Observed audio signals from an indoor experiment and (b) separated signals by ICA.

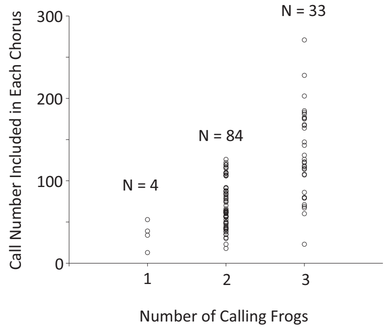

Fig. 7. Size effect on call number. From the audio signals of 4 hours, we succeeded in detecting 57 choruses (chorus of a single frog: 4, chorus of two frogs: 42, chorus of three frogs: 11). Call number is calculated per chorus per frog.

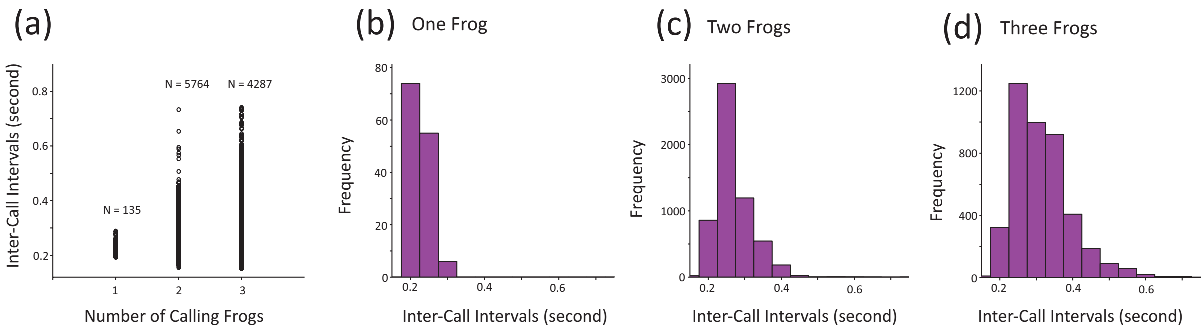

Fig. 8. Size effect on inter-call intervals. (b)–(d) represent the histograms of the intervals for different chorus sizes.

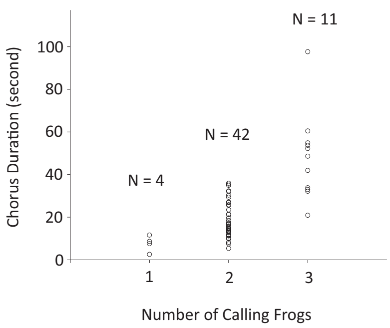

Fig. 9. Size effect on chorus duration. The chorus duration is calculated for each chorus.

demonstrates that the chorus size has a positive effect both on call number and inter-call intervals (call number: $\beta = 0.714 \pm 0.020$, $z = 35.61$, $P < 0.001$, inter-call intervals: $\beta = 0.130 \pm 0.004$, $z = 35.27$, $P < 0.001$).

Figure 9 represents the relationship between chorus size and chorus duration. The analysis using GLM shows that the chorus size has a positive effect on the chorus duration ($\beta = 0.931 \pm 0.118$, $z = 7.88$, $P < 0.001$).

4. Discussion

4.1. Behavioral Meaning

We have shown that call number, inter-call intervals, and chorus duration take larger values as the number of calling frogs increases. In general, female frogs tend to prefer call properties of male frogs deviating from the mean value [17]. We speculate the reason of our observation as follows:

1. The increase in the number of calling males makes their mating competition more severe.
2. Accordingly, male frogs change their call properties so as to make themselves more attractive to conspecific females.

However, it is known that female frogs prefer shorter inter-call intervals (i.e., higher call rate) in general [17]. Therefore, it is unlikely that male frogs calling at longer intervals take advantages for mating. Further studies are required to determine the behavioral implication of the relationship between chorus size and inter-call intervals.

4.2. Relationship Between Synchronization Patterns and Inter-Call Intervals

In our previous study, we reported that inter-call intervals during anti-phase synchronization are longer than those during in-phase synchronization [25]. However, the analysis was based on just a small amount of chorus data. Here, we examine the relationship with a larger data size.

To quantify the synchronization patterns, we calculate phase difference between calls of Frogs n and m as follows [14, 26]:

$$\phi_{nm} = 2\pi \frac{t_{m,j} - t_{n,i}}{\Delta t_{n,i}}. \qquad (7)$$

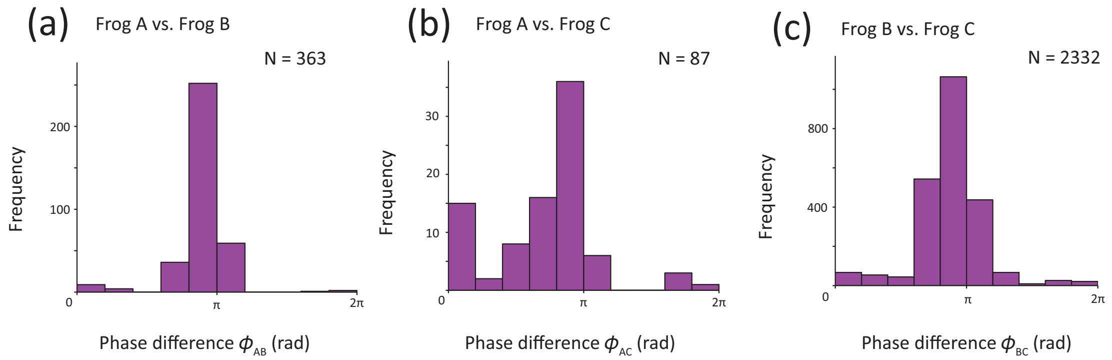

Fig. 10. Phase difference between calls of two frogs. There is an obvious peak at π, demonstrating that each pair tend to call alternately.

This phase difference is calculated when Eq. (6) and the following conditions are satisfied [14]:

$$n < m, \quad \ldots \quad (8)$$

$$t_{n,i} \le t_{m,j} < t_{n,i+1}. \quad \ldots \quad (9)$$

Consequently, ϕ_{nm} is restricted from 0 to 2π. Here, $\phi_{nm} = \pi$ means anti-phase synchronization of two frogs, while $\phi_{nm} = 0$ means in-phase synchronization of two frogs. Thus, we can distinguish synchronization patterns in frog choruses based on the value of ϕ_{nm}.

Relationship between the phase difference and inter-call intervals is then investigated. Here, we focus on choruses of two frogs for simplicity. The histogram of the phase difference ϕ_{nm} is shown in **Fig. 10**, demonstrating that there is a dominant peak at anti-phase synchronization for all the phase differences ϕ_{AB}, ϕ_{AC} and ϕ_{BC} while there is also a small peak at in-phase synchronization for ϕ_{AC}. **Fig. 11** shows the relationship between ϕ_{nm} and inter-call intervals. Note that the interval of Frog n is plotted in this figure when ϕ_{nm} is obtained. Although the intervals seem to take a larger value around $\phi_{nm} = \pi$, the scatter plot shows a complicated pattern. Future problems include the analysis using a statistical model on the scatter plot of two frogs as well as the analysis on the relationship between the inter-call intervals and synchronization states among three frogs (e.g., tri-phase synchronization and 1 : 2 anti-phase synchronization [14]).

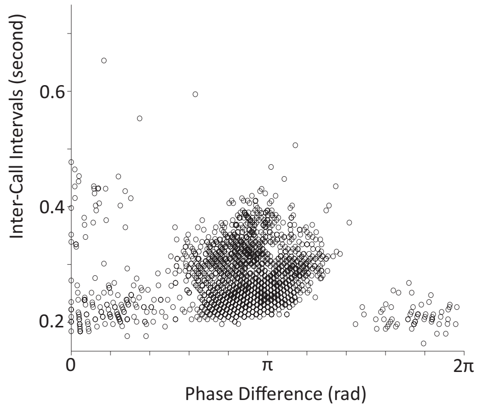

Fig. 11. Relationship between phase difference and inter-call intervals. The phase difference is calculated according to Eq. (7).

4.3. Individuality and Interaction in Male Frogs

Calling behavior of male frogs is affected by their individuality. In particular, it is known that the calling behavior requires high energy consumption [10]. Because the energy of individual frogs depends on their physical properties such as body size and weight that vary a lot, the relationship between the physical properties and call properties (e.g., call number and chorus duration) needs to be further examined. In addition, we need to analyze the effect of other call properties such as call frequency and call intensity that are not studied here. Such call properties are also related to the acoustic interaction among the male frogs, and can affect their behavior. Further studies are required to reveal the effect of such an individuality of the male frogs on their behavior and interaction, by repeating the similar experiments with different frog individuals.

4.4. Requirement of New Methods for Monitoring Complex Ecosystem

We demonstrate that calling behavior of male frogs fixed at stationary positions is discriminated by a stationary microphone array system. Future problems include the study on choruses of more individuals in their natural habitat where the frogs change their positions as well as occasionally overlap their calls. Sound-source localization in such a complex system is a more challenging task, because the performance of the localization would be deteriorated by the movement of animals as well as the call overlaps. To solve the first problem of the movement of animals, the development of a mobile robot equipped with a microphone is promising. To solve the second problem of the call overlap, we are developing a novel method for sound source discrimination that has a higher scalability, i.e., a sound-imaging device called *Firefly* [27]. The device consists of microphone and light emitting diode, and is illuminated when capturing nearby sounds. We deployed dozens of the devices along a ridge of a paddy field, and analyzed spatio-temporal structures inherent in the choruses of male Japanese tree frogs [27, 28]. We believe that the development of such a monitoring method allows us to reveal novel features of animal behavior in their natural habitat.

5. Conclusion

We have investigated the changes of call properties of male Japanese tree frogs in relationship to their chorus size. ICA allows us to discriminate calls of respective frogs. Analysis using statistical models has shown that a chorus size (i.e., the number of calling frogs) has a positive effect on call number, inter-call intervals, and chorus duration. Future problems include the study on chorus patterns of many frogs in their natural habitat. It is expected that the development of novel recording methods, e.g., auditory mobile robots, would contribute to the study of such complicated acoustic systems where a larger number of animals dynamically change their positions.

Acknowledgements

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