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Effectiveness of Mating Call Playbacks in Anuran Call Monitoring: a Case Study of Three-striped Pond Frogs (*Rana nigromaculata*)

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Abstract: We studied the effectiveness of mating call playbacks on call monitoring of three-striped pond frogs (*Rana nigromaculata*). Playback experiments were conducted between 2100 to 0030 at 15 sites located at Chungwon, Chungbuk, and Yeongi, Chungnam, in May 2005. We recorded call responses of 25 males to two different call playbacks, (i) single mating calls from a single male and (ii) chorus mating calls from five males, by randomly presenting the calls with a 15 min-gap between the two playbacks. We compared the number of response calls of the focal males for three min before, during, and after the playbacks. Five of 25 males were silent before stimulus presentation, but all the males emitted calls after the playbacks. The number of calls in response to single playback calls significantly differed among the three playback periods, where males gave more calls during the playback than before or after the playbacks. In addition, subject males presented significantly more calls to single call playbacks than to chorus call playbacks. The results of this study suggest that playback using repeated single mating calls is effective in call monitoring of *R. nigromaculata* males.

Key words: Mating call, chorus call, anuran call monitoring, *Rana nigromaculata*, three-striped pond frog

Amphibian populations have markedly declined in many parts of the world over the past several decades (Alford and Richards, 1999; Houlahan et al., 2000). To understand temporal and spatial variations of the populations and to elucidate causations of such decline, elaborated population monitorings have been conducted at local and regional levels. Data from the monitorings were used to conserve amphibian populations and to reverse decline trends in local populations. Amphibian populations in a wide area have been monitored mainly based on volunteer call surveys (McDiarmid and Donnelly, 1994). Since anuran call monitoring should be executed during limited times, from one hour after sunset to midnight, several weaknesses exist in the method. Also, species-specific daily and seasonal variation in calling activity may result in inaccurate assessment of a population size (Bridges and Dorcas, 2000).

The use of stimulated playback calls for monitoring a population has been used for many avian species, but little information is available for call surveys of anuran species. Many male anurans respond to playback calls in several ways: 1) by switching from advertisement calls to encounter or aggressive calls, where the males may have a call repertoire (Awbrey, 1978; Brenowitz, 1989; Rose and Brenowitz, 1991; Brenowitz and Rose, 1994); 2) by altering call features with a graded aggressive fashion, resulting in increased call duration (Wells and Taigen, 1986; Klump and Gerhardt, 1987), call repetition rate (Gerhardt, 1988), call rate (Márquez, 1995), call complexity (Wells and Schwartz, 1984), and high amplitude (Wagner, 1989); or 3) by matching calls with the playback calls (Gerhardt et al., 2000). Such behavioral changes of male anurans in response to playback calls could be used as a monitoring tool by increasing the probability to detect individual males in potential breeding sites.

Until recently, there have been no systematic surveys for Three-striped pond frogs (*Rana nigromaculata*) populations. *R. nigromaculata* is a common species in most areas of Korean peninsula including Jeju Island. They breed in the vicinity of agricultural lands. The males mainly aggregate in a chorus at breeding sites in the evening although some of them often sing in relatively isolated breeding areas. Mating calls are very similar among individuals and composed of three to eight pulse groups separated by clear silent

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intervals. They respond in a graded aggressive fashion to the playback of conspecific mating calls with significant increases in the number of call groups, rate of pulse groups, and dominant frequency and with a decrease in the pulse repletion rate (N-1/pulse group duration × 100; where N = the number of pulses; Park and Yang, 1997).

In this study, we examined call activity of *R. nigromaculata* before, during, and after the playbacks of single mating calls and chorus mating calls. Specifically, we tested the following questions: (1) Do subject males increase the call activity in response to playback calls? (2) Are the responses to the single mating calls versus to the chorus calls different in the number of response calls?

MATERIALS AND METHODS

Playback experiments were conducted in 15 sites of the vicinity of Chungwon, Chung-buk, and Yeongi, Chung-nam, in May 2005. To produce playback tapes, mating calls of *R. nigromaculata* were recorded in a population of the study sites with a Marantz PMD222 tape recorder and condenser microphone (type MKH 816 P48, AKG c1000s). Playback tapes for the experiments were prepared with Raven 1.2 (Cornell Laboratory of Ornithology 2004) with sampling rate 44.1 kHz and Cool Edit Pro. 1.5 (Adobe® Audition™ software). A playback tape contained mating calls from a single male, refered to as a single playback call hereafter, and the other tape contained mating calls in a chorus (chorus playback call) (Fig. 1). We chose only high quality call parts in a series of call utterance for both playback tapes. While the chorus playback call consisted of mixed calls from five males sang at close distance within 10 m, the single playback call was made of repeated calls with a single call type from one individual (Fig. 2).

We tested 25 males of *R. nigromaculata* between 2100 to 0030 on days of fairly good weather conditions, avoiding days with heavy wind or rain. We placed the speaker (JBL-Pro III) about 5 m away from the edges of the ponds towards the occupied populations, which was connected to a Sony portable minidisc recorder (MZ-R700). The call intensity from the speaker varied between 95-110 dB (Larson-Davis Lab. Model 800B) at 50-70 cm. It was 10-20 dB higher than that of the natural calls. Before conducting each experiment, we monitored the sites at least twice to make sure the existence of *R. nigromaculata* males. The two types of playback calls were presented in random orders with 15 min gap between trials. We recorded the number of calls of the subject males for 3 min before, during, and after the playbacks.

Since we knew from the preliminary research that the 15 min gap was enough for the subject to show independent responses to each playback call, we compared the changes in call numbers among the three periods of playbacks

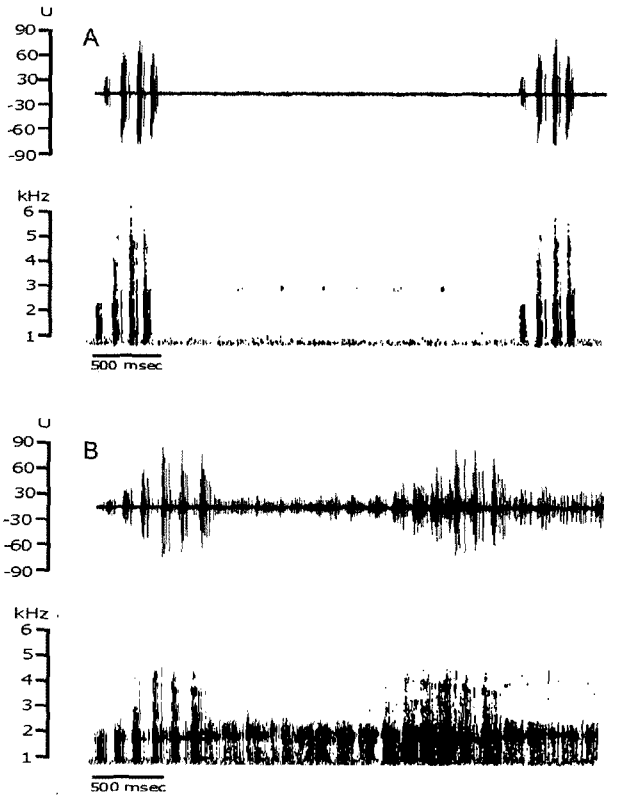

Fig. 1. Parts of *Rana nigromaculata* mating calls used for playbacks. (A) Two single mating calls with a 2.5 sec inter-call interval from a single male. (B) Two mating calls from different males, a part of chorus mating calls from five males. Oscillogram is depicted above the sonogram.

within each playback call type and then compared call changes of subject males to single and chorus mating calls during and after playback periods. Data were analyzed using SPSS statistical software (v.11.5; SPSS 2002). As the data did not meet normality assumptions of parametric statistical tests (One-Sample Kolmogorov-Smirnov Test, $p < 0.05$), we used Friedman test, the nonparametric equivalent of a one-sample repeated measures design, to compare call activity of focal males among the three playback periods within each call type. Then, we used Wilcoxon singed ranks test, the nonparametric equivalent of a paired t-test, to compare the call effects by a single playback call versus a chorus playback call during and after playback period. Numerical data in the text were presented as mean ± SD.

RESULTS

Five of 25 males initially did not emit any mating calls before conducting playbacks, but all the males gave calls in response to playback calls. We excluded the data of the five males in the further analyses.

The number of calls emitted by subject males to single

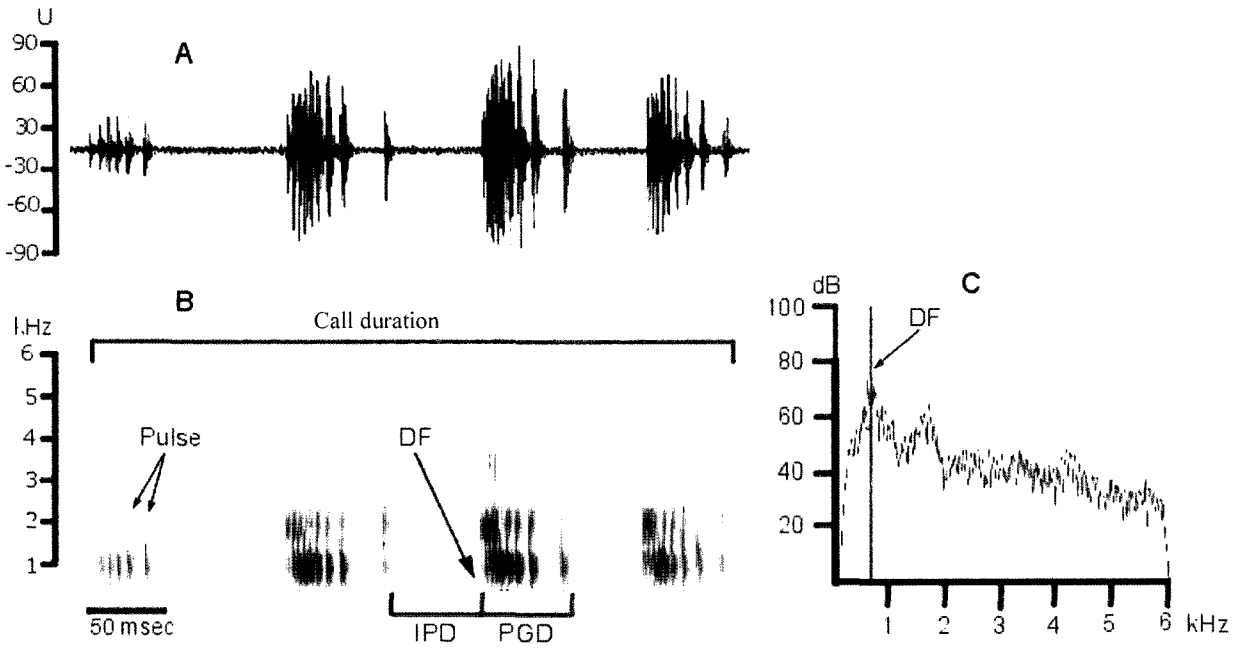

Fig. 2. Mating call structure of *Rana nigromaculata* used as a single playback call showing oscillogram (A), sonogram (B), and spectrum (C). A call consists of 4 pulse groups with average pulse group duration (PGD) 62.3 msec, 0.46 sec call duration, 63.7 msec inter-pulse intervals (IPD), and 786 Hz dominant frequency (DF).

playback calls significantly differed among the three playback periods (Friedman test; $\chi^2 = 6.911$, df = 2, $p = 0.032$), and were highest during the playback trials (Fig. 3A). However, no significant differences in response to the chorus playback calls appeared among the playback periods ($\chi^2 = 1.481$, df = 2, $p = 0.477$; Fig. 3B).

In addition, males gave significantly more calls to the single playback calls than to the chorus playback calls during the playbacks (Wilcoxon Singed Ranks Test: $Z = 2.593$, $n = 20$, $p = 0.01$; Fig. 4A), not after the playbacks ($Z = 0.598$, $n = 20$, $p = 0.55$; Fig. 4B).

DISCUSSION

The present results indicate that a playback method using single mating calls is efficient in call surveys of *R. nigromaculata* males. Playbacks of the mating calls induced call utterances of silent resident males and the other males responded with higher calling activity in the presence of the stimulated mating calls.

The increased calling activity of *R. nigromaculata* males could be induced by intruder effects within the subjects' territories, where the males significantly changed temporal and spectral structures of the mating call except for the total call duration: 1) by decreasing interval between pulse groups; 2) by increasing the number of pulse groups per call; and 3) by lowering dominant frequency (Park and Yang, 1997). The modified call parameters in response calls without significant changes of the total call duration may lead to an increase in the number of pulses emitted per unit time. As a result, higher call detectability per unit time due to increased call frequency as well as increased pulse number within each call induced by mating call playbacks would provide more opportunities to identify and locate anuran males in breeding sites.

Call playbacks of single mating calls may be a useful tool in call surveys of anuran amphibians in three ways: First, the call playback could improve assessment of the accurate population size by facilitating call activity of male frogs. Second, it could save time and energy to monitor wide-ranging areas. Playbacks could be easily performed in a variety of frog habitats that are often difficult to have a close access to. Third, the tool could be properly developed as a general field protocol for other species, particularly those who show high daily variations of call activity. For example, mixed types of playback calls were also efficient for monitoring Gold-spotted pond frogs (*Rana plancyi*), an endangered species in Korea, even in the daytime (H. C. Sung, unpublished data). Further research on species-specific acoustic behaviors is needed to apply the playback techniques to other species.

Single mating calls induced more call response from the resident males than chorus calls did. Such differential responses of male *R. nigromaculata* to the two types of playback calls have behavioral salience. First, resident males reduced or shifted call timing responding to the chorus calls to avoid call overlap in a chorus (Schwartz, 1993; Grafe, 1996; Greenfield and Rand, 2000) because the

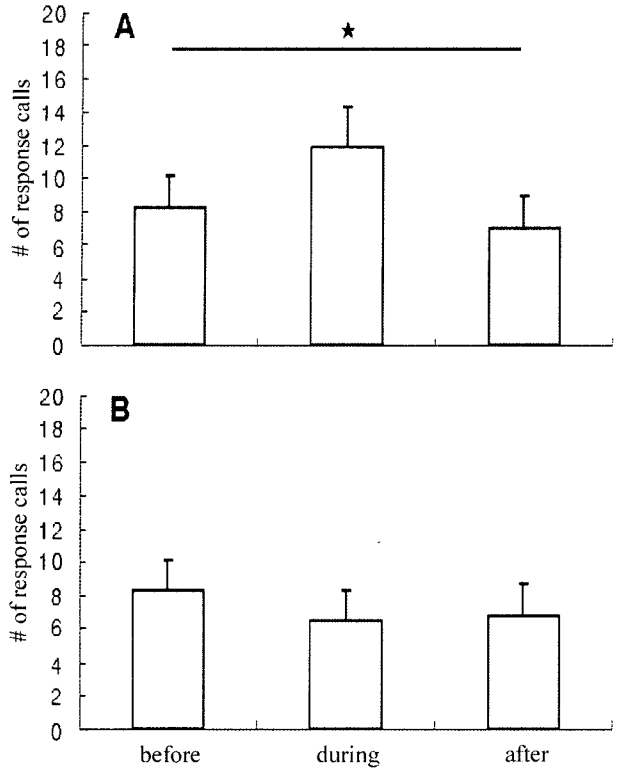

Fig. 3. Comparisons of calling activity (mean ± SE) of *Rana nigromaculata* among three playback periods in response to a single playback call (A) and to a chorus playback call (B). *$p < 0.05$ from Friedman test.

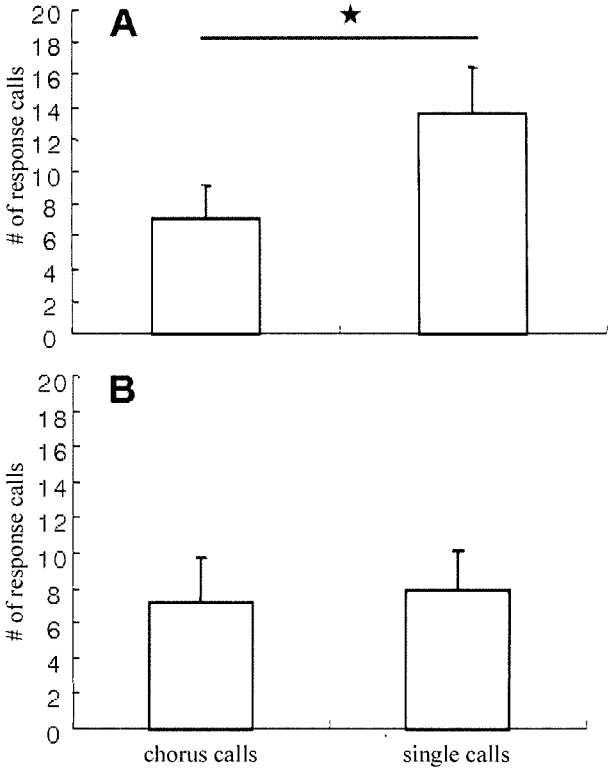

Fig. 4. Comparisons of calling activity (mean ± SE) responded to chorus and single playback calls in *Rana nigromaculata* during the playbacks (A) and after the playbacks (B). *$p < 0.05$ from Wilcoxon singed ranks test.

call overlap makes it more difficult to transmit the mating call in larger choruses than in smaller choruses. Second, males may increase call duration to maintain males' relative attractiveness in chorus size (Schwartz et al., 2002; Kime et al., 2004), possibly resulting in decreased call frequency. Schwartz et al. (2002) showed that male gray treefrogs decreased average call duration corresponding to the decreased number of males in a chorus but maintained their relative call duration by rank. They suggested that the calls retaining relative positions in a chorus could be used as an honest indicator of good genes. Longer calls were favored by female gray treefrogs (*Hyla versicolor*, Wells and Taigen, 1986) and cricket frogs (*Acris crepitans*, Klump and Gerhardt, 1987). However, the call duration of male *R. nigromaculata* recorded during the stimulus period did not differ significantly from that recorded during the non-stimulus period (Park and Yang, 1997).

No previous information is available for *R. nigromaculata* on the changed call features by chorus calls and on female responses to call playbacks. We do not know at the moment why the chorus calls did not intrigue higher calling activity during the playback trials in this study. We can just infer that either call overlap is too severe to use in a dense chorus or long calls to overcome acoustic interferences are too expensive to produce under the selective pressure to maintain relative positions. Thus, to fully understand the acoustic interactions among males in a chorus, we need to perform further playback experiments in different sizes of chorus and to investigate the female preferences on call characteristics in *R. nigromaculata* males.

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