What's the “sexiest” time to call- temporal changes in the calls of an amphibian assemblage at Maliau Basin Study Center premises

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Abstract
We investigated the frog call intensity in two anthropogenic habitats at Maliau Basin Study Center premises. Two species, *Rana nicobariensis* and *Bufo juxtasper* dominated the two sites. The calling intensity of the studied frog communities peaked at 2200hrs. *Bufo juxtasper* tends to call more often under rainy conditions. In the presence of more calling individuals, *Rana nicobariensis* tends to use longer calls. The frog community at the more disturbed site tolerated anthropogenic disturbances.

Introduction

Acoustic signaling between animals involves a rather remarkable cooperative communication channel. It enables rapid transfer of information over a considerable distance in which the sender and receiver need not be in visible contact, such as through dense foliage or in total darkness (Capranica, 1977). Most frogs use vocalizations to synchronize reproductive behaviour between males and females (Gerhardt, 1988) and to mediate social interaction between males (Ryan, 1991). In some species, males use separate aggressive and attractive communication signals in these two contexts. In other species, males use a single dual-function communication signal that both repels rival males and attracts females (Berglund *et al*, 1996).

Vocalization of frogs may depend on and be influenced by several natural and anthropogenic factors, and anuran vocalization is a valuable tool for studying their temporal activity patterns, once the call of the species under consideration has been determined. Frog calls can be heard from a considerable distance, and often the species can be identified easily by simple listening.

Based on these factors, we investigated whether there's a temporal difference in inter-specific and intra-specific calling patterns of a frog assemblage around Maliau Basin Study Center (MBSC).

Materials and Methods

Study sites

We conducted the study in two sites within the study center premises of the MBSC. “Site A” was ~25m^2 waterlogged area with a fallen tree near a newly constructing building, hence the site was not disturbed during the night. The other site (“site B”) was a larger size grassy patch adjoining the cafeteria and was regularly disturbed from human activities during then night. The variation of rainfall and light availability during the sampling sessions are given in Table 1.

Table 1. The variation of rainfall and light availability during the sampling sessions at the two sites.

Conditions / **Time (hrs)**	**Site A**		**Site B**	
	Rainfall	**Light**	**Rainfall**	**Light**
1800	Light shower	Dim day light & artificial light	Light shower	Dim day light & artificial light
2000	Heavy rain	Artificial light	Light shower	Artificial light
2200	-	Artificial light	-	Artificial light
0000	-	Artificial light	-	No light

Field sampling
We conducted field experiments in four sessions between 1800 and 0015hrs at two hour intervals on 22 and 23 August 2007, and during each sampling session we recorded two replicates of frog calls for two minutes each using a Marantz PMD660 professional recorder. All recordings were done placing the recorded at the same position. Furthermore the light condition (as dawn, dark but with lights and dark-without lights) and the weather (as clear, light showers, heavy rain, windy etc.) were recorded for each sample. Additionally, in some occasions, attempts were taken to actively search for the frogs using a maglite to confirm the call patterns.

Analysis
In the lab we analysed the calls using Expstudio Audio Editor. The number of "call parts" (parts of a complete call, e.g. as three for a call as "*kek kek kek*") were counted for each species at each site for the respective replicate. We entered data using OpenOffice Calc and performed subsequent analyses using program R 2.5.1. To visualize the overall temporal vocalization fluctuations, we plotted the variation of the mean number of call parts against time at the two sites (figure 1) and the mean number of call parts produced by the two species during the two days at different sampling times (figure 2).

ANOVA on the mean number of call parts at each time of the night was performed. We also used chi square test to compare the calling composition (*Rana nicobariensis* and *Bufo juxtasper*) between day 1 and day 2 in each site at each time.

Observations and Results

Four sympatric species of frogs, *Rana nicobariensis* (cricket frog), *Bufo juxtasper* (giant river toad), *Fejervarya limnocharis* (grass frog) and a *Philautus* sp. shared the two temporary waterholes near artificial lighting and clear, distinct calls could be only identified from the first two species. *Rana nicobariensis* was the most common species in the two sites and were always found submerged or afloat in water or sitting on land on the edge of water. Its call comprised six to eleven sharp "*kek*" notes in a rapid sequence. *Bufo juxtasper* which was present at both sites had a hoarse, squeaky croak. Being a species which prefer grassy areas *Fejervarya limnocharis* was only observed at Site B while due to the lack of any suitable perching platforms, the *Philautus* sp. was restricted to site A.

The mean number of call parts at each time of the night showed a clear pattern of increasing till 2200hrs and subsequently decreasing, at both sites (Fig. 1). *Bufo juxtasper* showed a significant difference in the number of calls between the two days.

According to the chi square test the calling composition (number calls from the two different species) at site A showed a significant difference ($p<0.001$) between the two days at 1800, 2000 and 2200 hrs. But site B only showed a significant difference ($p<0.001$) at 2000 hrs with respect to calling composition.

Being in a regularly disturbed site, the frogs at site B did not showed any changes in the calling upon approaching them for recording, but the frogs at site A completely stopped calling upon reaching ~5m to the site and only started calling after 30-45 seconds.

We also detected a relationship between the number of call parts of *Rana nicobariensis* with the number of individuals calling. During 1800hrs when few individuals were calling the average call parts ("*kek*") of an individual were six while during 2200hrs when the calls were very high, the average call parts per individual were 11.

Discussion

The anuran call is now widely considered an important character for species identification, more distinctive than many morphological characters. Its function of attracting conspecific females makes the mating call the primary species specific isolating mechanism in anuran speciation (Zimmerman & Bogart, 1988). Sympatric species have distinct calls if they are closely related to each other. Selection will favour acoustic divergence where two species with similar calls are sympatric (Bogert, 1960). As the mating calls of a species are always highly characteristic, they are useful criteria for the determination of interspecific and intraspecific relationships (Schneider *et al,* 1986). Though much work has been done on the frog calls with respect to its function (eg. female attraction, males repellance), very few studies have been done on the temporal differences of calling.

Due to the lack of sophisticated recording equipment and bio-acoustic software, the number of call parts could be only estimated. The counting was further affected by acoustic interference. However, the problem of acoustic interference in large choruses has been solved in similar ways by frogs and many insects. In both the groups, males tend to space their signals so that those of one individual do not overlap those of its near neighbour. In some species, call alternation is very precise and may lead to the formation of duets, trios, or other call groups within a large chorus (Kanamadi, 1994).

The decrease of the callings after 2200hrs (Figure 1, graph D of figure 2) may not be based on the unavailability of artificial light, as the same pattern was repeated both days. However, artificial lighting may effect the activities of frogs by, 1) providing easier access to food from the insects attracted to artificial lights at night, and 2) by aiding visual communication. The higher number of callings of *Bufo juxtasper* during the first day may have been due to the showers.

The complete silence or reduction of calling volume of frogs at site A can be due to the lack of continuous exposure to anthropogenic disturbance which may result in the males employing a bluffing technique, in which they either completely stop of lower the dominant frequency of their call. Frogs are also know to employ this condition in response to hearing the lower frequency call of an intruder. Given that the call frequency is negatively co-related to the body size, bluffing may provide a means for avoiding displacement by an intruder male. A study by Sun & Narins (2005), illustrated that anurans are sensitivity to anthropogenic noise pollution and some species exhibit rather novel calling behavior in response to a complex and dynamic acoustic environment. But according to our observations the populations at site B seems not be disturbed by continuous human activities happening around the site.

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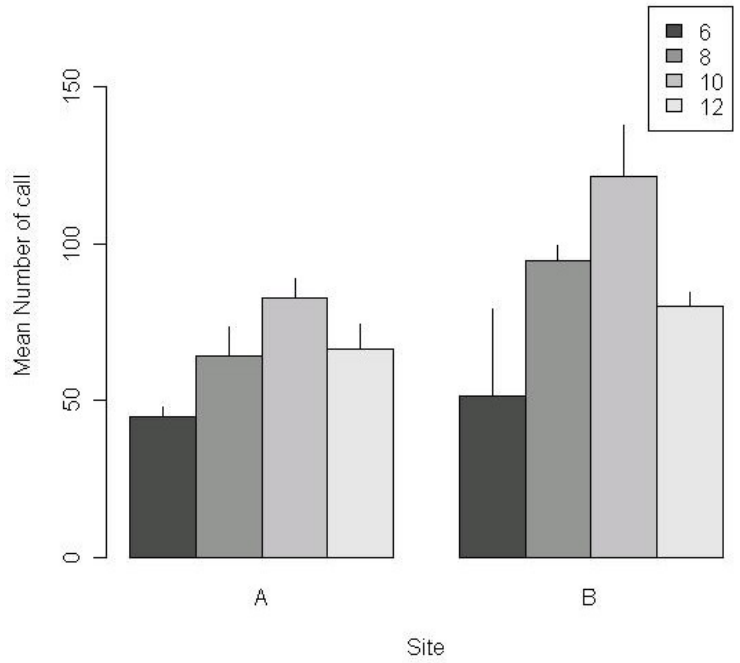

FIGURE 1. Mean number of calls in each time of the day.

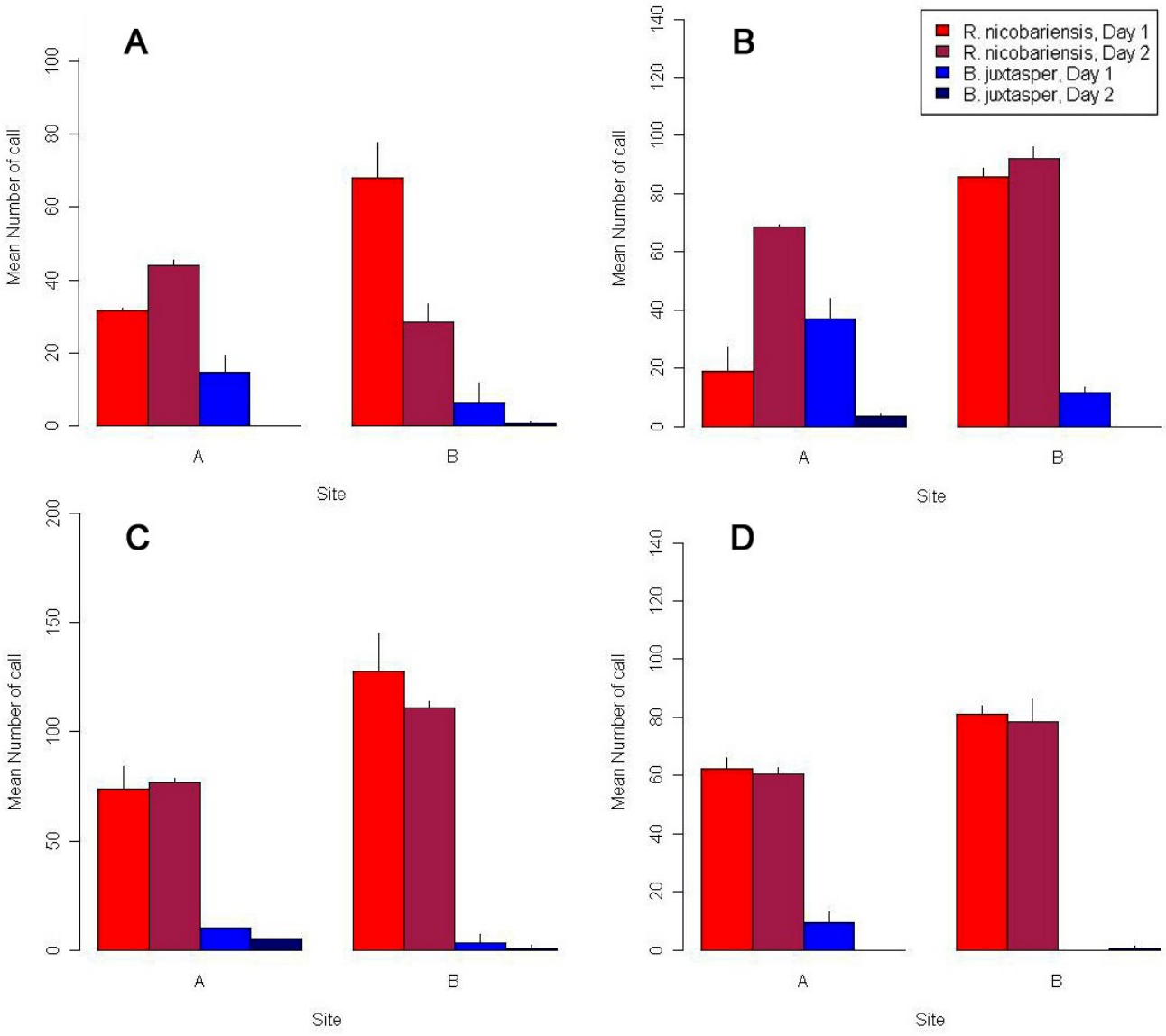

FIGURE 2. Mean number of call parts at each site of each day. *A*- at 1800hrs, *B*- at 2000hrs, *C*- at 2200hrs and *D*- at 0000hrs.