Study of Intermale Spacing in Calling Males of the African Reed Frog, *Hyperolius puncticulatus* (Pfeffer, 1893), in Amani Pond.

by

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ABSTRACT

Intermale spacing in calling males of the African reed frog, *Hyperolius puncticulatus* (Pfeffer, 1893), was investigated in Amani pond. Call-amplitude, -frequency, and -rate were investiagetd for the possible effects they might have on male spacing. The study showed that there is definite spacing of calling males in the pond, as determined by nearest calling neighbour distance. This distance could however not be explained by any of the call parameters investigated in the study. The distance between nearest calling neighbours range between 36cm and 332cm. Most calling males (62% of individuals counted on 11-13 August) exhibited fidelity to call site in the study.

INTRODUCTION

One of the most characteristic night sounds in Amani, Tanzania, is that of calling frogs. Acoustic cues, along with visual and scent-based ones, have evolved by natural selection to represent species-specific communication modes. Thus whereas people talk (cry, laugh, and scream as well), dogs bark, lions roar, and frogs croak. The question is: why do frogs 'croak'?

On the whole, frog calls are used for mating purposes. Although frogs may emit an array of other sounds (aggressive calls, release calls, and distress calls) they mostly emit advertisement or mating calls (Passmore & Carruthers, 1995). Therefore, specific mate recognition systems (SMRS) of anurans are centred on this acoustic signalling. As a direct consequence of this, different frog species have different mating calls, thereby making calls the most important premating reproductive isolation mechanism between species (Hodl, 1977). Frog calls are therefore highly reliable characters upon which to base the diagnosis of species (Passmore & Carruthers, 1995). Typically, the calls of amphibians are most evident when they breed (Halliday, 1996) making breeding aggregations (leks) invaluable communities in which to carry out investigations.

Wignarajah and Marques (1999) encountered six species of frogs from the genus *Hyperolius* and one from *Afrixalus* in the Amani pond, in the year preceding this study. Their study revealed that there was a clear relationship between the colour of the plant site at which the hyperolid frogs were located during the day and their dorsal colourations. Their study organisms were *Hyperolius parkeri*, *H. spinigularis*, and *H. mariae*, but not *H. puncticulatus*. Another study in the pond by Durrans & Riva, 1998, investigated the incidence of call station height preferences among the different frog species inhabiting the pond. The findings were that no significant preference for any particular calling height existed among the hyperolids, whereas *Rana angolensis* (Common river frog) seemed to call at a lower height than the others. This is not particularly surprising as the hyperolids are climbers, whereas the river frogs are hoppers and leapers, and have no discs for climbing, or to allow them to hold on to plant parts above the water surface. The study also revealed that there was no relationship between frog size (as determined by snout-vent length) and the height at which it called.

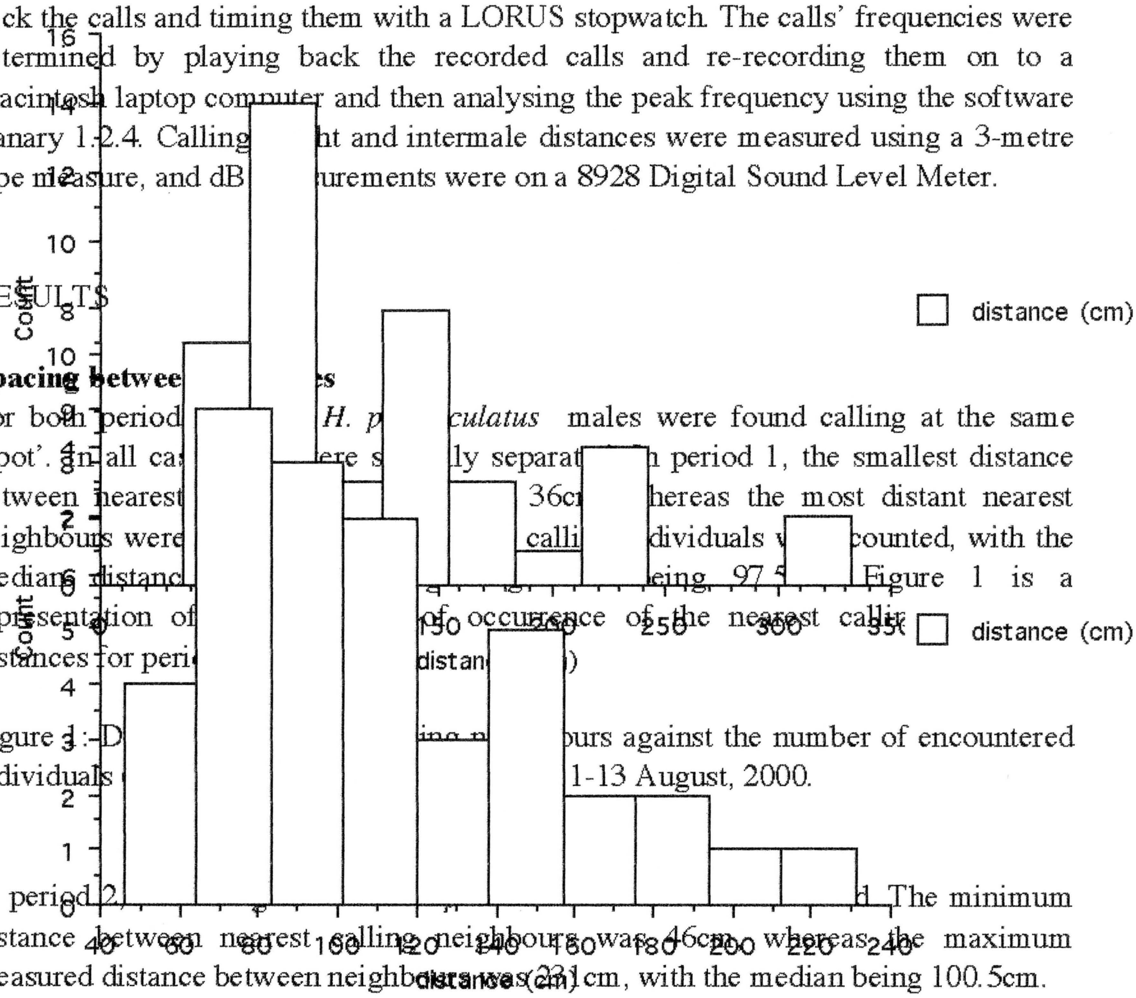
This study attempts to investigate spatial distribution of calling males of *H. puncticulatus* . This frog species is 'by far the most abundant frog in Amani pond and one of the most numerous in the Usambara mountains' (Verstergaard, 1994). Also, the study investigates possible factors that might influence spacing between the calling males: call height, frequency, rate and amplitude.

METHODS & MATERIALS

The study site, the reed-bed in Amani pond, was measured and a rectangular plot 18metres by 12metres selected, as it had the best reed-bed coverage. There was no scientific basis for making the plot rectangular except for ease of sampling. The plot was further divided into square quadrats of side 3 x 3 metres. Each of the 24 quadrats was sampled twice within the study period. Duplicate sampling of the quadrats meant that most frogs were encountered more than once. The two complete surveys were split into periods 1 (11-13 August) and 2 (15 & 16 August).

Calling activity of *H. puncticulatus* was monitored in these quadrats of *Cyperus papyrus* -dominated habitat for five nights. Pond sampling time was at night from about 1830 hours to 2230 hours and was carried out by the two authors. During the course of the study, the site from which a *H. puncticulatus* was calling was located by navigating towards the call source and undertaking a search for the calling individual(s). On observing the calling individual, a recording of the call was made, an amplitude (dB) measurement taken at a horizontal distance of 50cm from the calling frog. The calling height was simultaneously measured, and the site marked with a ribbon (on the reed from which the frog calls) and correspondingly mapped on a map having the different quadrats for later identification. A dorsal sketch of each individual was made as a rough guide to identifying the frog at a later stage. The marked sites were revisited on each subsequent day, and the intermale distance between markings of the two nearest neighbours measured. Nearest neighbours were not arbitrarily decided but measurements were made for all marked sites surrounding one particular site, and the determination for nearest neighbour determined later from the collected data.

Calls of *H. puncticulatus* used in analyses were not recorded throughout the study period, but only on the nights of the 15th and 16th August. Recordings were made on a SONY three head cassette-recorder TCM-5000 EV using an external SENNHEISER microphone; the microphone was in all instances held horizontally from the calling male at a distance of about 30 centimetres. Call rates were determined by playing back the calls and timing them with a LORUS stopwatch. The calls' frequencies were determined by playing back the recorded calls and re-recording them on to a Macintosh laptop computer and then analysing the peak frequency using the software Canary 1.2.4. Calling [illegible]ht and intermale distances were measured using a 3-metre tape measure, and dB [illegible]urements were on a 8928 Digital Sound Level Meter.



RESULTS

Spacing between [illegible]s

For both period[illegible] *H. p*[illegible]*culatus* males were found calling at the same 'spot'. In all ca[illegible]ere s[illegible]ly separat[illegible]n period 1, the smallest distance between nearest [illegible] 36c[illegible]hereas the most distant nearest neighbours were [illegible] calli[illegible]dividuals [illegible]ounted, with the median distance [illegible]eing 97.5[illegible] Figure 1 is a representation of [illegible] of occurrence of the nearest callin[illegible] distances for peri[illegible]

Figure 1: D[illegible]ing n[illegible]ours against the number of encountered individuals [illegible] 1-13 August, 2000.

In period 2, [illegible]d. The minimum distance between nearest calling neighbours was 46cm, whereas the maximum measured distance between neighbours was 231cm, with the median being 100.5cm.

Figure 2: Distances of nearest calling neighbours against the number of encountered individuals (count) of *H. puncticulatus* on 15 & 16 August, 2000.

A coefficient of dispersion (CD) test was carried out to investigate whether the occurrence of calling *H. puncticulatus* frogs in the pond was random; i.e., were the distances between nearest calling neighbours independent events with no relationship? The CD values were 40.702 and 16.948 for periods 1 and 2 respectively, indicates that the distribution is clumped (Sokal & Rohlf,1981), meaning that the occurrence of one frog depends on the location of other frogs.

Relationship between nearest neighbour distance and call rate, frequency and amplitude.

The correlation between nearest neighbour distance and call rates, frequency and amplitude were investigated. Table 1 shows the descriptive statistics for these parameters and Table 2 the results of Spearman's rank tests between these nearest neighbour distance and these variables.

Table 1: Summary of the measurements obtained for amplitude (dB), frequency of recorded calls, and call-rate analyses in *H. puncticulatus.*

Descriptive Parameters	Amplitude (dB)	Call rate (calls/min)	Frequency (kHz)
Mean	81.6756757	19.5176923	3.33974286
Median	81.8	18.18	3.358
Mode	81.4	17.65	3.36
Standard Deviation	3.33328378	4.37473576	0.10901736
Variance	11.1107808	19.138313	0.01188478
Range	14.1	23.07	0.4925
Minimum	74.5	13.85	3.0925
Maximum	88.6	36.92	3.585
Sum	3022	761.19	116.891
Count	37	39	35

For the Spearman's rank correlation test presented in Table 2, nearest neighbour distance was used as the second variable in all cases. The test shows that there is no statistical correlation between each of the parameters investigated, and male spacing.

Table 2: Spearman's rank test of nearest neighbour distance as a function of call parameters in *H. puncticulatus.*

Parameter tested	P- value
dB	0.6978
Frequency	0.7772
Call rate	0.1259

Site fidelity

A degree of site [illegible]: 61.9% of th[illegible]ered in period two were found in th[illegible] in period 1). The distances with [illegible] where they were originally sighted [illegible] range of 0 - 56 centimetres from each other. These distances and number of frogs (count) are shown graphically in Figure 3.

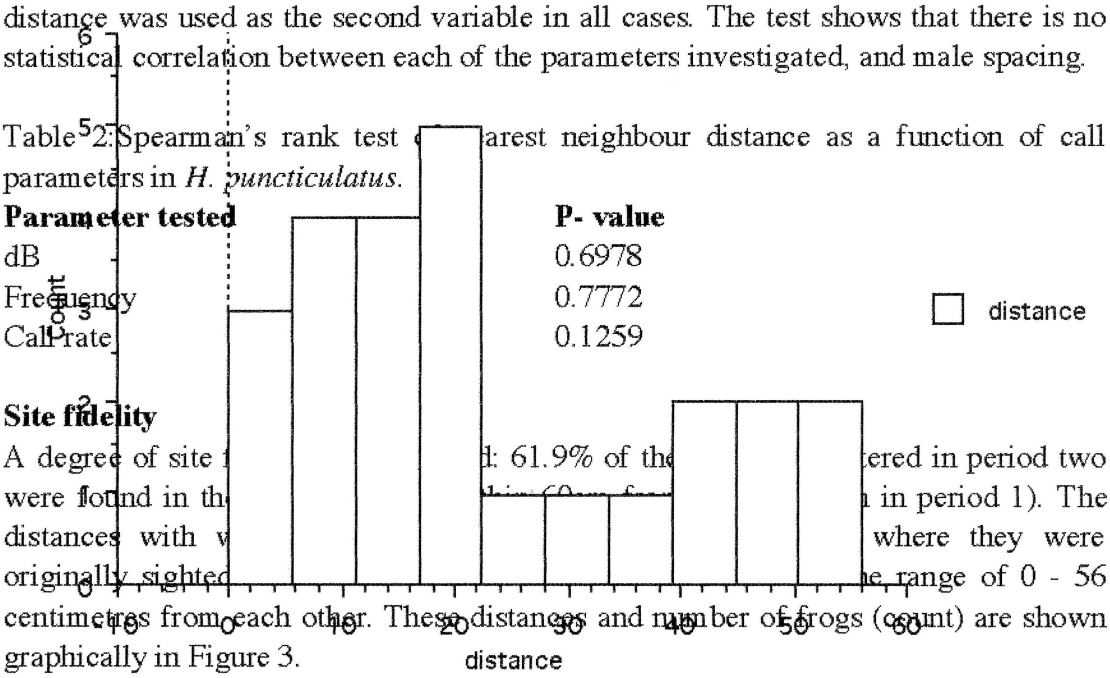


Figure 3: Distance between *H. puncticulatus* that re-occurred in the same vicinity between 11-13 August and 15-16 August, 2000.

A Wilcoxon signed rank test was carried out, pairing for each frog (that re-appeared in the same vicinity in period 2), the distance that it moved from the original site, and the difference between distances of nearest calling neighbour in both periods. This was to test whether there was conformity in the distances by which frogs would move from their original calling sites, and the adjustments they would make in the intermale spacing with their nearest calling neighbours. A p-value of 0.6997 was obtained, indicating that the distances moved were to effect adjustment so as to keep distances to nearest neighbour more or less constant. The same test was done for the heights at which the individuals called from in the two periods. The p-value for this was 0.5203, indicating that there is no significant difference between the heights from which the frogs call in the two periods.

DISCUSSION

Male spacing

Hyperolius puncticulatus in the Amani pond reed-bed are not randomly distributed, but clumped in distribution. This means nearest calling neighbours are somehow influencing each other's position. Hence, calls emitted by neighbouring individuals that stand out against the environmental noise level are likely to be of communicative value in the intermale spacing of *H. puncticulatus*. . A study on natterjack toads, *Bufo calamita*, revealed that calling males were only able to assess calls from nearest conspecific individuals but not from distant ones, while failing to detect the presence of satellite (noncalling) males present in their immediate vicinities (territories) (Krebs & Davies, 1994). This lends support to the phenomenon of nearest calling neighbours influencing each other's distribution in *H. puncticulatus*, thereby assigning some operational value to the minimum intermale distancing.

Observations in Amani pond, indicate that the peak spacing distance range is between 60cm-90cm for periods 1 and 2. The mean nearest neighbour distances were 125.7cm (period 1) and 110.6cm (period 2), but were not significantly different. For purposes of minimal spacing distance between callers, the mean is not an an adequate measure, neither is the median nor the mode. An investiagtion seeking to identify such a distance ideally should investigate frog behavioural responses to proximity of calling conspecifics.

Fidelity

Having established the existence of an operational inter-male spacing, analyses for consistency in intermale spacing for the different nights were made. Evidence of site fidelity was detected: 61.9% of the males counted in the first period were present in the second period at the same calling sites. These frogs exhibiting fidelity to calling sites also maintained similar nearest neighbour distances, adjusting these by moving minimally from their original positions. Movement was to sites at similar heights. These observations seem to support the earlier findings of non random spacing of calling males in the pond. Therefore whereas there have been observations of high male turnover in aggregated males during breeding periods, there seems to operate parallel to this a high level of site fidelity with adjustments whenever the intermale spacing distance is altered by the arrival (or most probably the return) of other males. It seems likely that some operational (optimal) intermale distancing was taking place in Amani pond.

Call parameters influencing intermale spacing

Calls of *H. puncticulatus* were observed to be separated temporally though this was not quantified. Nearest neighbours spaced their calls so that they did not call at the same time. Whenever two or more consecutive calls coincided, one of the callers would seemingly delay a call so that it came at intervals to its neighbour's calls. Passmore and Carruthers (1995) call this sequential ordering of calls by neighbouring males 'antiphony'. The assertion that all *H. puncticulatus* frogs at a site call at the same time and then go quiet (Stewart, 1967) is not supported by our study.

Several other call parameters were also analysed in an attempt to investigate their effect on intermale spacing. What influences the distancing aspect of intermale spacing in *Hyperolius puncticulatus*? Female frogs that breed at night have been found to navigate towards calling conspecific males using calls with no reliance on either sight or smell (Passmore & Carruthers, 1995). Playing back recorded calls to females of *Hyla regilla* from loudspeakers had them navigating to these sound sources and nudging them just as they would do males in nature (Krebs & Davies, 1994). Therefore an attempt was made to identify the aspect of the call that best explains intermale spacing as this would most likely confer frogs with some competitive advantage in mate attraction.

*Call amplitude determinations revealed that there was no appreciable difference in the call amplitudes of the individuals in the pond; amplitude also had no noticeable relationship with nearest neighbour distances. This would make sense for aggregating frogs, as the loudness of a call is a very subjective character, depending on the location of the intended reciepient. It can be masked by the environment, such as a dense growth of reeds. Also females will pass near all calling males at least once. Hence, energetically, sustaining very loud calls must be expensive, and in a pond where the approach direction of the female is not certain, the loud caller is not very much advanatged by his call. Hence the pond frogs seemed to choose an optimal amplitude for their calls as this is cheaper. Selection seems to favour such signals that strrike an optimum balance between greater effectiveness and lower fitness cost (Johnstone, 1997)

* Call frequencies for the *H. puncticulatus* males were not significantly different. The mean, mode, and median frequencies all coincided for the sample at 3.34kHz. Statistical analyses showed that the frequency was not correlated to the intermale spacing distance.

* Call rate, measured as the number of calls emitted per minute were determined for frogs encountered in period two. The call rates were not associated to the intermale spacing distances in the pond. However, the P-value of 0.1229 makes call rate an important parameter to carry out more studies on, using a bigger sample and over a longer duration. Noteworthy from the raw data on call rates is that the two highest (and four out of the six highest) call rates were from individuals that were less than 60cm from their nearest neighbours. These frogs elevated their call rates by emitting 'double-calls'. Whereas ordinarily calling males would make one call and then be quite for a reasonable duration before making another call, double-callers would emit two simultaneous calls separated only briefly by a 'breathing pause' before going into the quite phase. It is therefore probable that call rates may play a

role in intermale distancing (in combination with some other factor(s)), hence double calls may be used when frogs become close to each other and are employed as a spacing/competitive mechanism.

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ACKNOWLEDGEMENTS

Our sincere gratitude is extended to the following individuals who assisted us during this study: Dr Mike Cherry, Dr Barrie Goldsmith, Dr Rosie Trevelyan, Proff Bruno Bruderer and all other TBA partcipants who contributed to the production of this chapter.

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