Xenopus laevis (Anura: Pipidae) Mating systems – A preliminary synthesis with some data on the female phonoresponse

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The mating system of hormonally stimulated *Xenopus laevis laevis* has been examined. The mating call of the male can be divided into two parts, one displaying a greater pulse repetition rate. These two components follow one another in calling bouts without intervals of silence between them. Temporal characteristics of the call are presented. The mating call has a complex frequency structure. Up to five energetic frequency bands are present, and individual frogs from the same population emphasize different bands. Female *Xenopus laevis* respond to the mating call with a positive phonotactic response. This has been quantified, and the associated behaviour described. Females, when clasped by males, produce a release call which rarely resulted in the release of the female, the result of overstimulation of the male with sex hormone. Males give a soft 'amplectant call' while clasping. Clasped males produce a release call which effects their release. Of all modes of communication, the mating call is considered to convey the highest information content for mate recognition. Other communication relates to the physiological condition of the clasped frog.

S. Afr. J. Zool. 1980, 15: 150 – 158

Die paringsstelsel van hormoongestimuleerde *Xenopus laevis laevis* was ondersoek. Die paringsroep van die mannetjie word in twee dele verdeel waarvan die een 'n groter trilherhalingstempo toon. Hierdie twee komponente volg mekaar sonder stiltes tussen die roepbeurte op. Temporele eienskappe van die paringsroep is beskryf. Die paringsroep het 'n ingewikkelde frekwensiestruktuur, met tot vyf energiebande. Indiwiduele paddas van dieselfde bevolking beklemtoon verskillende bande. Die wyfie *Xenopus laevis* reageer positief fonotakties op die paringsroep van die mannetjie. Dié is gekwantifiseer en die gepaardgaande gedrag is beskryf. Wanneer wyfies deur mannetjies vasgegryp word, produseer hulle 'n vrylatingsroep, wat as die klopgeroep beskryf kan word, maar as gevolg van die oorgestimuleerde geslagshormone van die mannetjie het dit selde die vrylating van die wyfie tot gevolg. Die mannetjie gee 'n sagte 'ampleksieroep' terwyl hy vasgryp, maar gee 'n manlike vrylatingsroep wat vrylating tot gevolg het. Van alle soorte kommunikasie bevat die paringsroep maksimum inligtingsinhoud vir paarherkenning. Verdere kommunikasie het betrekking op die fisiologiese toestand van die vasgegrypte padda.

S.-Afr. Tydskr. Dierk. 1980, 15: 150 – 158

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Received 30 November 1979; accepted 10 March 1980

Xenopus laevis (Daudin) is maintained and routinely induced to breed in laboratories throughout the world, but despite this, its reproductive behaviour remains incompletely understood. Its suitability as a laboratory animal resides largely in the ease with which it can be kept in the laboratory, and made to breed with the use of sex hormones (Shapiro 1936a, 1939; Russel 1954; Nieuwkoop & Faber 1956; Gurdon 1967; Kelley & Pfaff 1976).

Bles (1901) described the breeding of *X. laevis laevis* (see also Bles 1906; Vanderplanck 1935; Haubrich 1961; Deuchar 1975) and, although the mating behaviour has since been investigated by a number of authors, no synthesis has yet been made. None of the calls have been investigated in terms of their function, not all of the calls have been noted, and only a few have been studied quantitatively. In this paper some new aspects of *X. laevis* mating behaviour are examined. The calls have been given a functional basis, and in one case the function has been tested experimentally.

The fact that *X. laevis* calls underwater has been noted by Schmalhausen (1957), Bushnell (1957) and Bogert (1960). Müller and Scheer (1970) noted three different calls of *X. l. laevis:* a call given by the male while in amplexus, a release call given by most amplectant females, and another call given spontaneously by isolated males. Sughrue (1969) described the release call of the female, and a tonic posture given by most amplectant females, where the body is stretched out stiffly (See also Grimm 1952). Noble and Aronson (1942) also noted this tonic posture (in *Rana*), and found that it was the thinness of the stretched out female that caused the male to release her. The female release call was inhibited if the frogs were injected with saline to simulate ripe ovaries. Russel (1954) described a low frequency tremor given by males when in amplexus with females. This behaviour was also noted by Grimm (1952). Although many authors have provided verbal or phonetic descriptions of the calls of *Xenopus laevis,* only two have thus far published sonograms, *viz.* Müller and Scheer (1970) for the three calls of *X. l. laevis;* the release call of the female, the mating call of the male, and a call given by solitary males. Vigny (1979) has sonographed the call of 12 species and subspecies of *Xenopus,* and summarized the acoustic characteristics of each. (The species and subspecies concerned were: *X. l. laevis, X. l. petersi, X. l. victorianus, X. borealis, X. clivii, X. fraseri, X. gilli, X. muelleri, X. ruwenzoriensis, X. tropicalis, X. vestitus,* and *X. wittei.*)

Many authors have observed that in a number of anuran species males and females respond to conspecific calls, and are thus attracted to the breeding site. This topic has been adequately reviewed by Salthe and Mecham (1974). There is one anecdotal reference to a positive phonotactic response by a female *Xenopus* (Sughrue, 1969). In the paper which follows the female phonotactic response has been investigated quantitatively.

Materials and Methods

A large number of *X. l. laevis* was obtained from a commercial dealer supplying animals from Fish Hoek (southern Cape Province), and others were seine-netted from a pond at Plettenberg Bay, 450 km east of Fish Hoek (Cape Province). The frogs were housed in plastic tanks (262 ℓ capacity), and were fed macerated liver once per week. The water temperature ranged from 19—22 °C. In order to avoid overcrowding, only 15—25 frogs were housed per tank (overcrowding is known to reduce the sexual activity of the females (Zwarenstein & Shapiro 1933; Shapiro 1935; Alexander & Bellerby 1935, 1938).

Only on rare occasions does *X. l. laevis* spawn naturally in captivity (Russel 1954). Sexual behaviour was therefore stimulated by subdermal injection of Pregnyl, a mammalian chorionic gonadotropin (Organon laboratories, Crown House, London Road, Surrey, England). This is generally accepted as being the only reliable method of breeding *Xenopus* in captivity (Shapiro 1936, 1939; Nieuwkoop & Faber 1956; Rusel 1954; Gurdon 1967). Full nuptial pad development was found to be a reliable indicator of sexual activity in males. The hormone doses required for stimulation of full sexual activity differed in summer and winter, frogs requiring a larger dose during the latter period. In summer a single dose of 400 I.U. Pregnyl was sufficient to induce sexual behaviour in males. Females with the abdomen distended with eggs and with hyperaemic labia required a single dose of 750 I.U. This generally ensured ovulation about eight hours after injection. During winter (April—August) males frequently required two courses of 400 I.U. Pregnyl each, given a week apart. Females required a primer dose of 300 I.U., and a further dose of 600 I.U. a day later.

All voice recordings were made in a darkened soundproofed room, using a red light for behavioural observations. Subjects to be recorded were isolated in 25ℓ aquaria. An Adastra Electret microphone (800 ohm impedance) was waterproofed in a thin rubber sleeve, and lowered into the water (Rabb & Rabb, 1960, have shown that there is no difference in the quality of recordings done with waterproofed microphones as opposed to hydrophones). The vocalizations were recorded with a Uher 4000 Report tape recorder at a tape speed of 19 cm/s. The calls were analysed on a Kay model 7029 A sonograph. The analysis was done in the frequency range 40–4 000 Hz. The water temperature at which the calls were recorded ranged from 21–24 °C.

Analysis of the female phonoresponse

Each trial comprised a group of five females. They were injected five hours before the trial so that their ovulation would coincide with the test period. Each group of females was put in the 0,7 × 0,7 × 1,8 m concrete test tank 30 min before the experiment commenced, so that they could acclimatize to the surroundings. A waterproofed speaker (5 cm diameter, 8 ohm resistance) was used for the sound playbacks on a continuous loop of the call of the male given during amplexus. The temperature at which the call was recorded, and the temperature of the water in which it was played back differed by 1–3 °C. The calls were played back at approximately the same volume as the call given by the live frog. Intervals of 30s silence followed every few minutes of playback, as Adrian, Craik and Sturdy (1938) found this to be more effective than playing back a continuous call. The tank was divided in half by a baseline, and the speaker positioned on the bottom, nearest the one side. When a stationary female was on the opposite side, the call of the male was played. The time taken for the female to reach the baseline was noted, as well as the time spent in the vicinity of the speaker. Usually the test was halted after the female had spent more than 3 min in the vicinity of the speaker. All recordings and observations were done in a darkened room, using a red light for observation.

Results

Male – female interactions

After hormonal treatment, mating calls were produced by isolated males and males in amplexus. Occasionally, isolated, untreated males produced mating calls (usually following a water or temperature change). The call of the male was given while the frog rested horizontally on the substratum (Vigny 1979), stood half vertically on the substratum, or swam. No movement of the floor of the

Table 1 Pulse rate data for the mating call of *X. l. laevis*

Slow component	N	$\bar{X}$ (Pulses/s)	Range (Pulses/s)	S
Population				
Fish Hoek	16	33	24,2 — 41,9	±4,419
Plettenberg Bay	10	31,7	26,5 — 36,3	±3,06
Combined	26	32,4	24,2 — 41,9	
Rapid component	N	$\bar{X}$ (Pulses/s)	Range (Pulses/s)	S
Fish Hoek	16	55,5	43 — 66,4	±5,7
Plettenberg Bay	10	52,8	49,6 — 62,8	±4,9
Combined	26	54,2	43 — 66,4	

S — standard deviation
N — number
$\bar{X}$ — mean

Table 2 Duration of S and R components of the mating call of *X. l. laevis* (R – rapid, S – slow)

Slow component	N	$\bar{X}$ (s)	Range (s)	S
Population				
Fish Hoek	16	0,530	0,34 — 0,8	±0,118
Plettenberg Bay	11	0,671	0,5 — 0,9	±0,203
Combined	27	0,60	0,34 — 0,9	
Rapid component	N	$\bar{X}$ (s)	Range (s)	S
Fish Hoek	16	0,307	0,2 — 0,6	±0,064
Plettenberg Bay	11	0,268	0,176 — 0,316	±0,05
Combined	27	0,287	0,176 — 0,6	

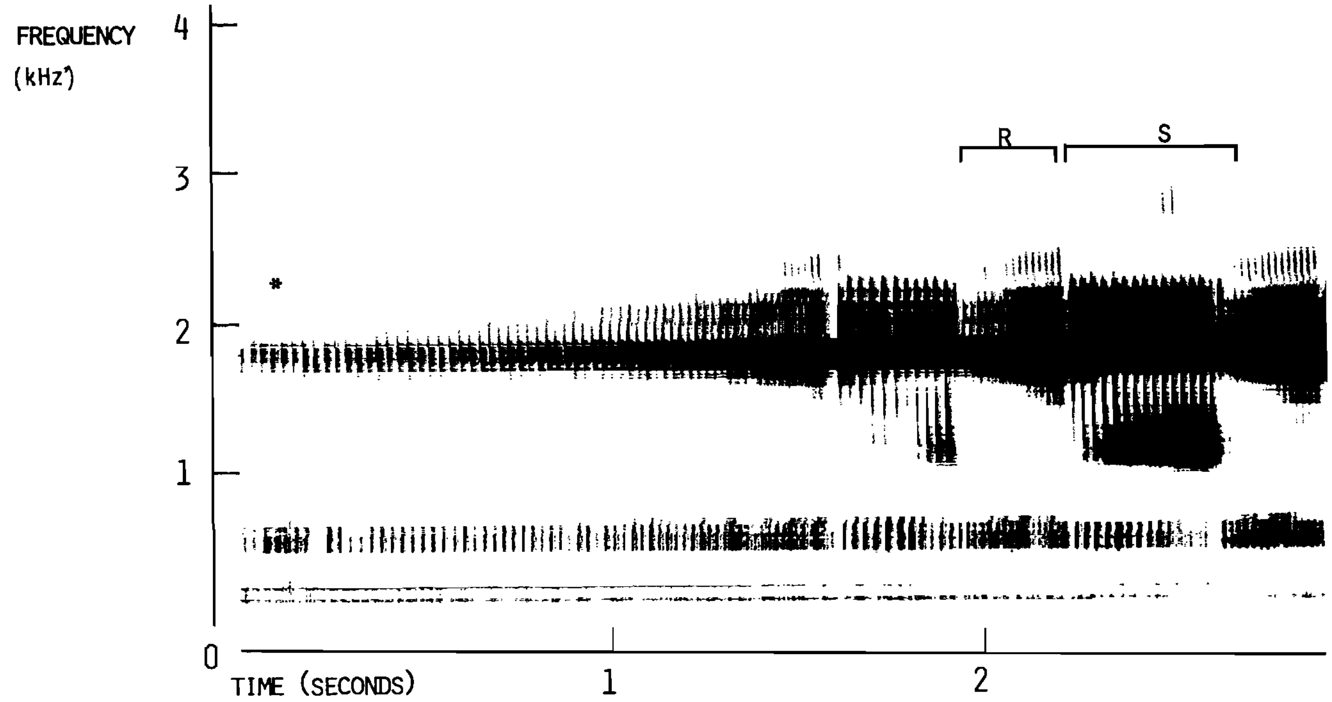

Fig. 1 Sonogram of the call given by isolated *X. l. laevis* male (mating call). R–rapid component, S–slow component (frequency range 40–4000 Hz, wide band. 300 Hz filter)*–start of calling bout.

buccal cavity was discernible (Grimm 1952). The mating call of the male was divided into two parts: one which had a greater pulse rate, here termed the rapid (R) component, and another component which had a slower pulse rate, here termed the slow (S) component. Table 1 gives the pulse rate data for the R and S components.

Calling bouts consisted of lengthy trains of alternating R and S components (Fig. 1).

Table 3 Frequency characteristics for the mating call of *X. l. laevis*. Band 5 was omitted from the analysis on account of its rarity

Frequency ranges for the five energetic bands:

Slow component

Band	Number of males displaying the band	X̄ (Hz)	Range (Hz)
1	6	85	60 — 149
2	10	918	774 — 996
3	10	1411	1125 — 1550
4	10	2191	1722 — 2361

Rapid component

Band	Number of males displaying the band	X̄ (Hz)	Range (Hz)
1	2	111	72 — 149
2	7	930	885 — 996
3	3	1678	1383 — 1973
4	10	2169	2084 — 2316

X̄ — mean

Calling bouts have been accurately described by Vigny (1979) as 'ion-ion-ion', etc., and continued uninterrupted for a number of minutes. Each bout began with an initial long drawn out R component (Fig. 1). The S component was of longer duration than the R component (Table 2).

This mating call had a complex frequency structure, and in some recordings up to five energetic frequency bands could be differentiated. Figure 2 shows a mating call with four energetic frequency bands present. The frequency characteristics for these bands are given in Table 3.

In the case of the R component however, the majority of the males emphasized frequency band four (Table 4). The mating call had a similar structure when given in isolation, or during amplexus. This call has been shown to attract females.

Response of the female to the mating call

Although many authors have termed the call given by isolated males the mating call, none have tested the function of this call. It was found to elicit a positive phonotactic response in five females. In each case the phonoresponse followed the same behavioural pattern.

After the mating call of the male had been played for

Table 4 Proportion of sample emphasizing the various frequency bands of the mating call

Rapid component					
Percentage of sample emphasizing the different bands					
	Band 1	Band 2	Band 3	Band 4	N
Population					
Fish Hoek	0	16,6	0	83	12
Slow component					
Percentage of sample emphasizing the different bands					
Fish Hoek	0	35,7	21,4	42,8	14
Plettenberg Bay	0	25	50	25	4
Combined	0	33	27,7	38	18

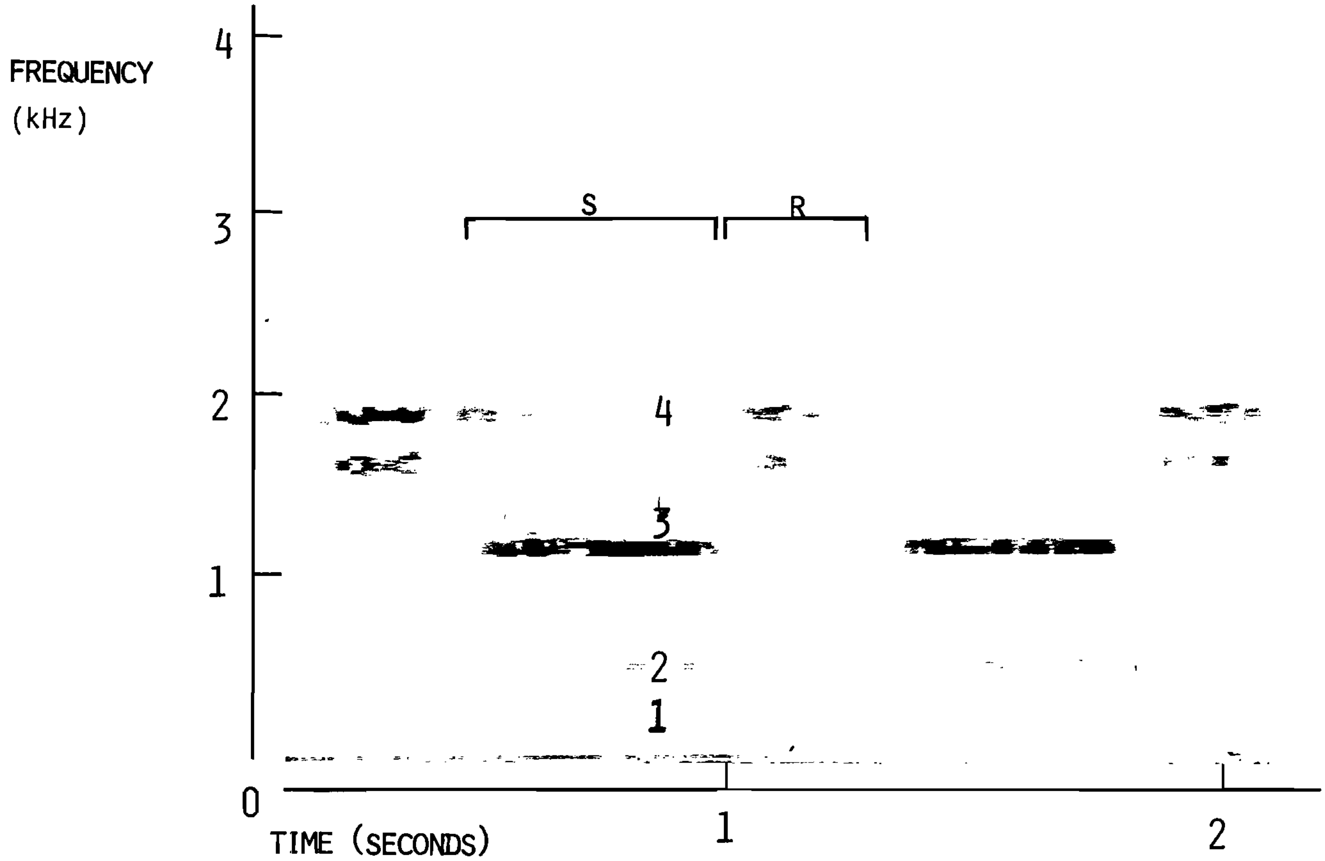

Fig. 2 Sonogram of call given by isolated *X. l. laevis* male (mating call) R—rapid component, S—slow component (frequency range 40—4000 Hz, narrow band, 45 Hz filter). Four bands are present in the S component (1—4).

about a minute, the female began swimming about. She then circled the speaker, with either the outside or inner arm slack, and folded back against the body. The diameter of the circles was then reduced progressively, until the female came to rest a few centimetres from the speaker (sometimes with the snout actually touching it). The circles range from 1,2 m —0,2 m in diameter. The latent period for the female perceiving the mating call before giving a locomotory response and the time spent in the vicinity of the speaker are given in Table 5.

Table 5 Wilcoxon 2-tailed matched pairs signed-ranks test for the female phonoresponse

Wilcoxon 2-tailed matched pairs signed-ranks test for the female phonoresponse.

Time spent at end of tank opposite speaker before moving to speaker (min)	Time spent at speaker (min)	D	Signed rank
2	2,5	+ 0,5	1,5
2,25	4	+ 1,75	4
2,75	3,25	+ 0,5	1,5
0,25	2,25	+ 2	5
0,8	2	+ 1,2	3
0,25	3,25	+ 3	7
0,8	3	+ 2,2	6
0,0	4	+ 4	8

T is significant at the 0,01 level for the 2-tailed test.

A Wilcoxon 2-tailed matched pairs signed-ranks test for these data gave a significant value at the 0,01 level. If another female swam past the responding female, the latter closely followed the former, even to the extent of altering her speed to keep pace. If the followed female stopped swimming, the pursuer indulged in feeding movements, here interpreted as displacement flicking (Russel 1954). Ovulating females gave an imperfect phonoresponse, since they circled, but did not stop at the speaker. Two females which had already ovulated followed another female, and clasped her loosely a number of times.

Only about 10% of all females tested gave a positive phonotactic response to the mating call of the male. The reasons for this are probably that some of the experimental animals had already ovulated, and others were still approaching the correct physiological condition for a phonoresponse. This is supported by the fact that only after one hour of continuous playback had some of the females begun to respond to the mating call.

In the small aquaria where the males and females were usually mated in captivity, the mating call of the male did not result in the approach of the female to the male, and clasping was a chance effect of the confinement of the animals. *Xenopus laevis* apparently have poor eyesight (at least at night) as females frequently bumped into a 7,5 cm diameter pipe, and the wall of the test tank. Observations indicated that the male was able to detect the turbulence caused by a passing female, probably through the well developed lateral line organs. On three occasions males were observed to ignore females swimming head on towards them. When, however, the female kicked out backwards on swimming away from or past the male, the male

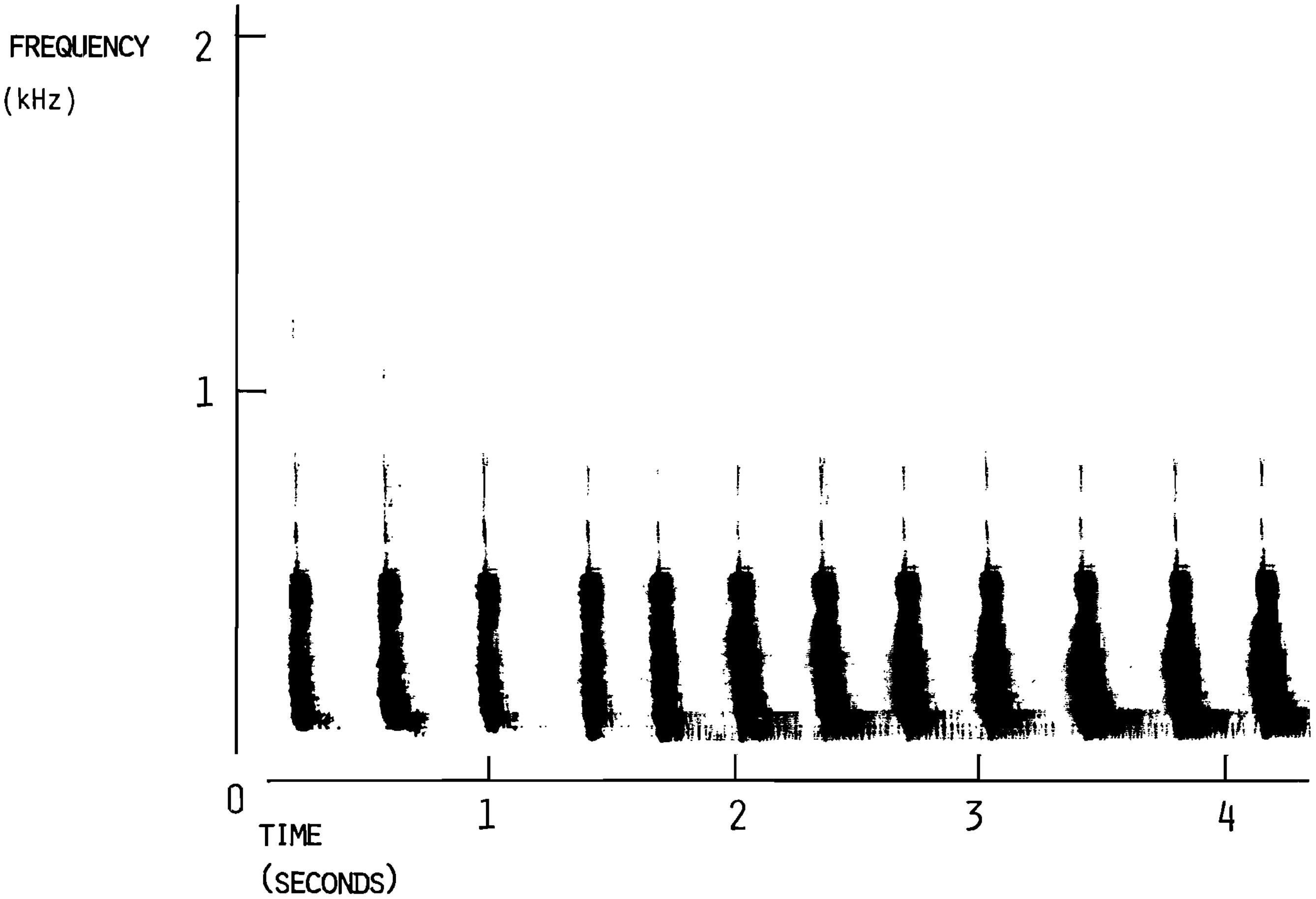

Fig. 3 Sonogram of release call given by female *X. l. laevis* during amplexus (frequency range 20–2000 Hz, wide band, 300 Hz filter).

immediately shot out and grabbed the female (initially by the thigh, but later adjusting his grip). In addition, some males, on sensing a frog in this way, swung around to face the frog's posterior region, and commenced calling. By any of the above methods, the male would come to clasp the female.

Amplexus and associated vocalizations

Males initially clasping the female's head or side usually soon released their grasp. Clasps on the hindlimbs almost always resulted in an inguinal amplexus, after some readjustment. In almost all cases the female gave the release call when grasped by a male (Fig. 3).

Only when the female had attained the correct physiological condition for ovulation to occur did she remain silent when clasped by the male.

Cases where an unreceptive, tapping female was clasped by a male not overstimulated with sex hormone resulted in the release of the female by the male. Females with a low level of sexual activity (i.e. those having received little or no Pregnyl) produced a louder call, with a greater pulse repetition rate (Fig. 4).

The structure of the call changed in the same way when a male altered a clasp from the legs to the waist (Müller & Scheer 1970). This tapping call could be given by a female breathing through the external nares. Many authors have assigned the function of a release call to this tapping call (Russel 1960; Poynton & Francis 1966; Müller & Scheer, 1970), although none have tested the function. Here the level of hormone treatment received by the male determined whether he would respond to the release call of the female or not.

Females stopped tapping once they had been released by the male. Females which had just been clasped and then released gave the tapping call if another frog then came into contact with them. The structure of the mating call of the male also varied depending on the behaviour of the female. If the female was passive when clasped, the male soon stopped producing the mating call. If the female tried to break loose when clasped by the male, the rate of the R and S components of the mating call increased (Fig. 5).

The mating call was most frequently delivered when the male clasped the leg of the female (this stage is one where the male could easily be dislodged). Once inguinal amplexus was achieved, the mating call of the male generally stopped.

The next sound made by the male has never been reported, although the behavioural movements associated with it have been noted by some authors. Russel (1960) referred to this as 'a low frequency tremor given by the male during amplexus, with the snout of the male depressed on the female's back'. The very soft call associated with this behaviour pattern is described here as the amplectant call. For each call the male flicked his head back a little and pressed his jaw against the back of the female. The male's body also shook slightly, even the toes, although it appears that the origin of this movement lay in the contraction of the jaw and forelimb musculature. The contraction was divided into three phases: a tightening of grip, arching of the back, and depression of the snout. Once amplexus was achieved, this became the predominant behaviour of the male (Fig. 6).

In cases where males were overstimulated with sex hormone, and did not respond to the release call of the female, the female would effect the release of the male by tapping louder and more vigorously, and at the same time

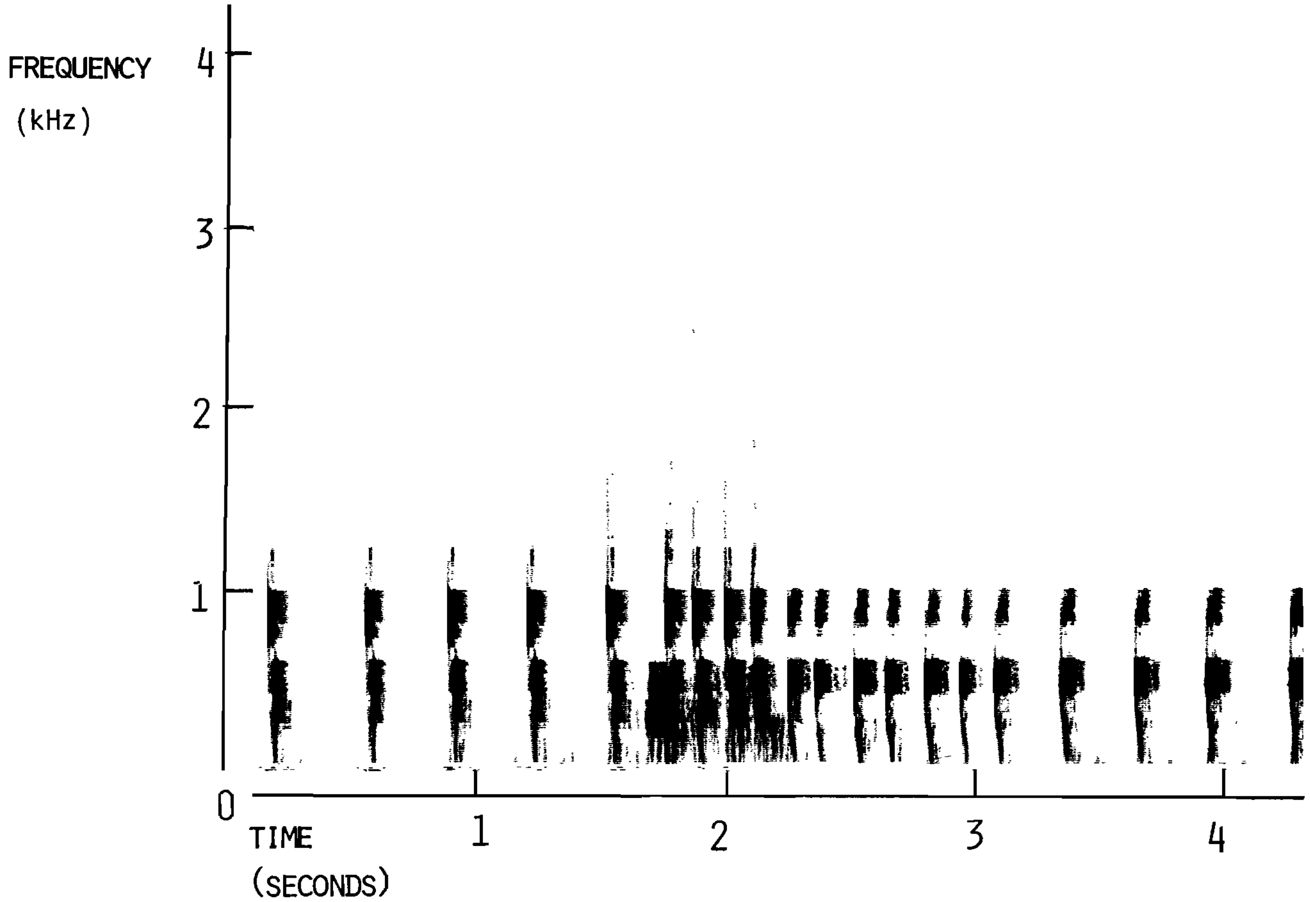

Fig. 4 Sonogram of release call given by resisting female *X. l. laevis* during amplexus (frequency range 20–2000 Hz, wide band, 300 Hz filter). Note the increased pulse repetition rate.

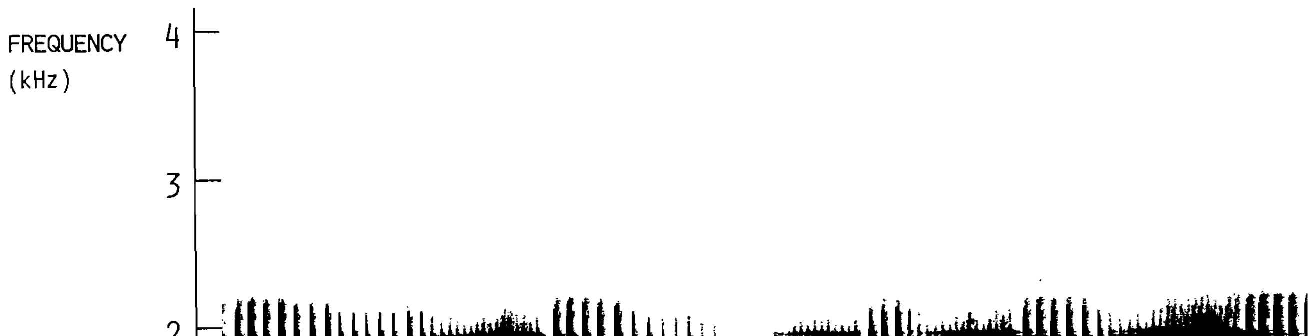

Fig. 5 Sonogram of mating call given by male *X. l. laevis* while in amplexus with protesting female (frequency range 40–4000 Hz, wide band, 300 Hz filter).

assuming an elongate, tonic posture accompanied by occasional kicks. The male generally released the female following a sudden, violent kick by the female.

Male – male interactions

Male *X. laevis* clasp other *Xenopus* regardless of sex. It therefore sometimes occurred that sexually active males clasped other males. In these instances the clasped animal uttered a growling call until it was released (Fig. 7). Clasped males were always released within two minutes.

The effect of Pregnyl

During winter (when animals were relatively unresponsive

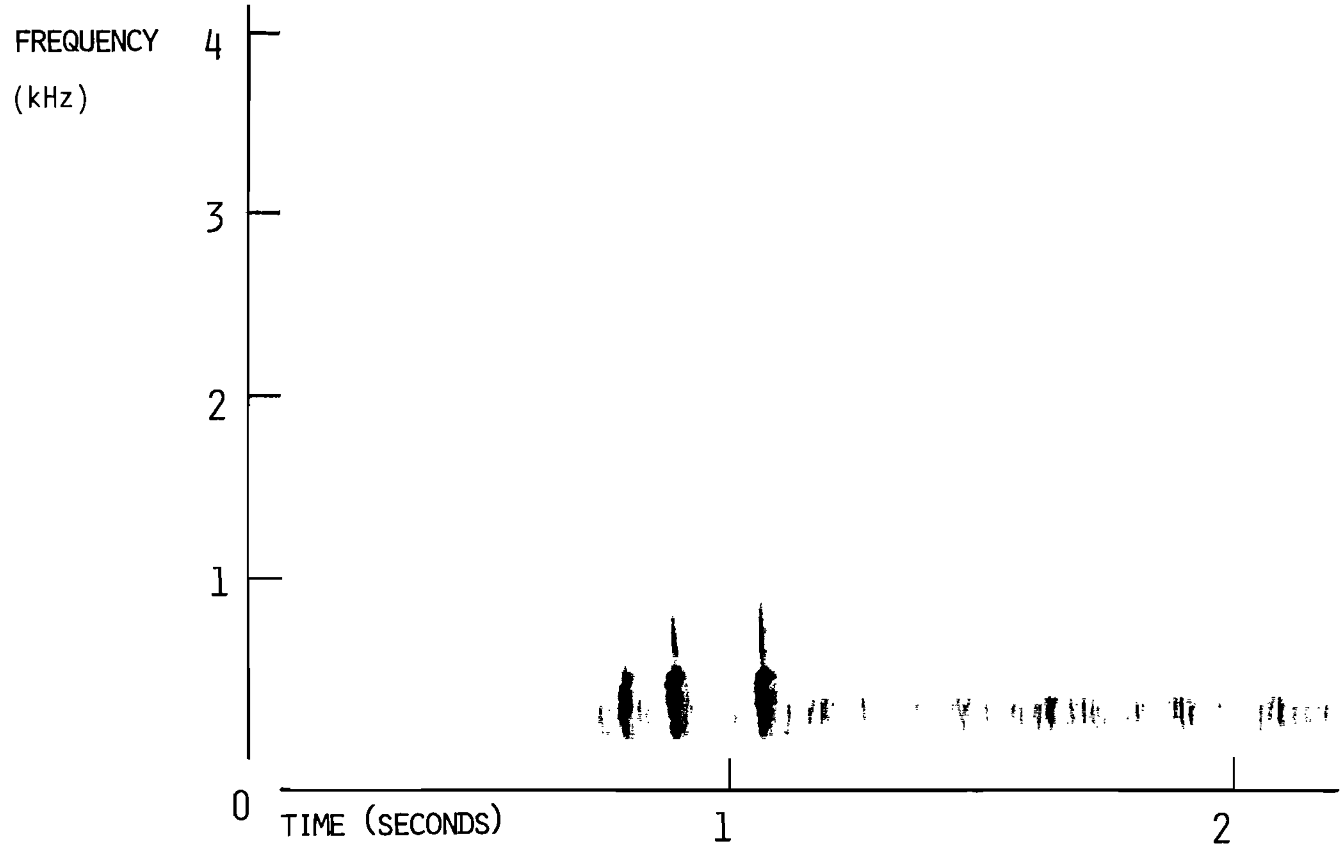

Fig. 6 Sonogram of the call given by male *X. l. laevis* during amplexus with passive female (frequency range 40–4000 Hz, wide band, 300 Hz filter).

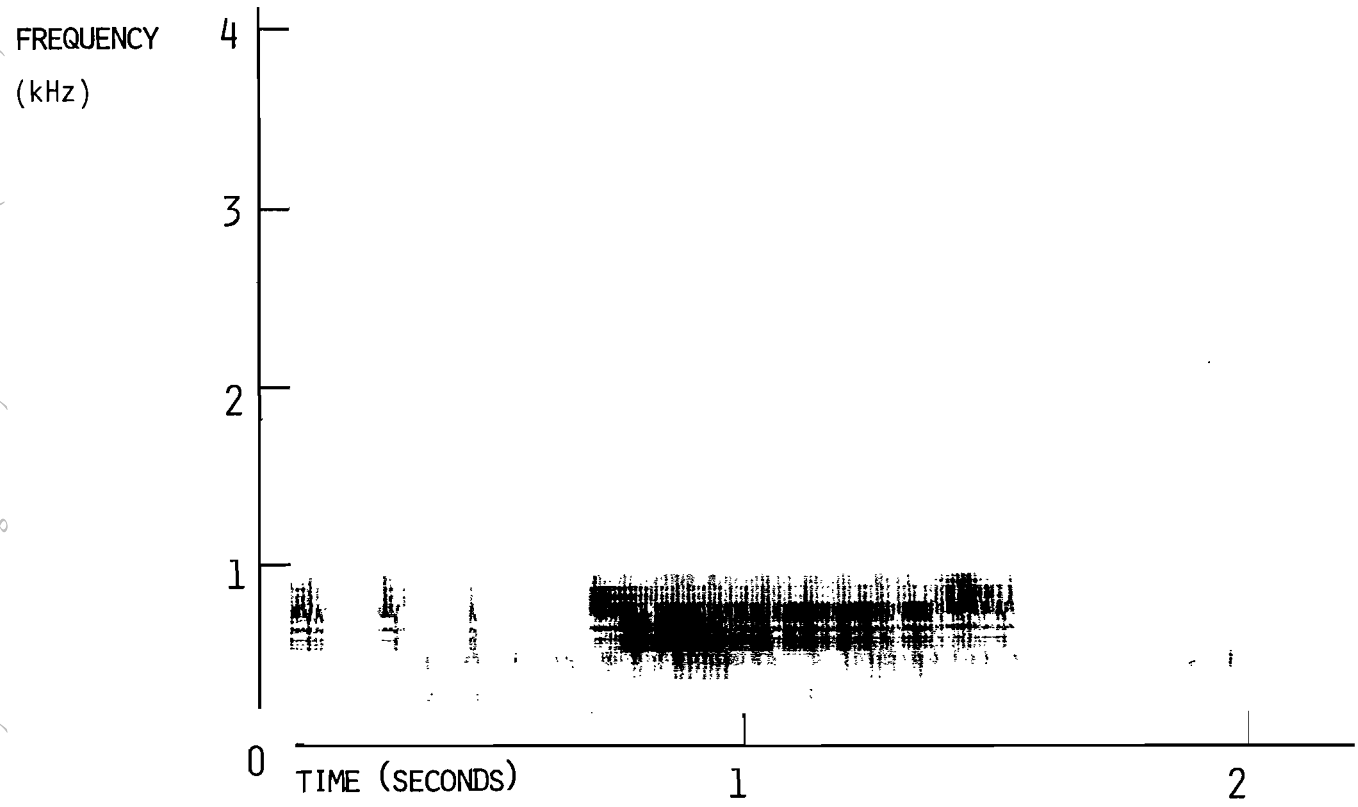

Fig. 7 Sonogram of the call given by male *X. l. laevis* while clasped by another male (male release call). Frequency range 40–4000 Hz, wide band, 300 Hz filter.

to hormonal treatment), a larger dose of Pregnyl was needed to induce sexual behaviour. In such cases a change from the normal mating pattern of the male was noted. A batch of nine males was treated with two consecutive doses of sex hormone. The resultant sexual behaviour was noteworthy in that in each case males achieved amplexus without at any stage having given the mating call. Normally a struggling female stimulated the clasping male to call, but these males remained silent, although sexually active and clasping normally.

Discussion

The need for a complex communication repertoire in *X. laevis laevis* becomes evident when one considers the breeding habitat of this animal. Although *X. laevis* is found in clear waters in South Africa, it shows a preference for turbid, plankton-enriched lentic bodies of water. Males and females, when in the correct physiological condition, must meet for syngamy to occur. Visual cues are probably unsatisfactory signals for mate location in this environment. Berk, Cheetham and Shapiro (1936) found that eyeless males suffered no reduction in mating efficiency. My observations in clear water suggest that night vision in *X. laevis* is poor, as animals would frequently collide with stationary objects in the observation tank. Any limited capacity for night vision would be further reduced in turbid water. The suggestion that night vision is not extensively employed in mate location is further supported by the presence of a complex acoustical repertoire, and the probable use of the lateral line organs in close distance detection of other frogs. Although *X. laevis* has no tympanum, this apparently does not reduce the frog's sensitivity to water-borne vibrations.

The call of the male *X. laevis* is not given in concert with others as a chorus for the following reasons. *X. laevis* generally live and breed in the same pond, and as a result males and females are always in close proximity.

All that is needed is for the males to produce a call which would attract conspecific females already in the pond directly to them. This would be more energetically efficient than reliance on chance meetings. Once females have approached males in response to their mating call, the lateral line organ of the male would detect the turbulence produced by the female swimming past. Males would then clasp the nearby frog (irrespective of sex). If in the unlikely event of the clasped frog being a male, or an unreceptive female, the release calls given ensure that no further energy is wasted in such fruitless clasps. Although further communication between males and females takes place, it is suggested here that the mating call of the male carries the highest information content for mate recognition. This is supported by the following: when confined in small aquaria, where the function of the mating call of the male is effectively eliminated, (i.e. no phonoresponse occurs), and chance collisions are inevitable and frequent, hybridization with resultant viable offspring occurs between many species. Vigny (1977) reports hybridization of this sort between a number of *Xenopus* species. Other communication between partners relates not so much to mate recognition, but to the recognition of the actual physiological condition of the mating partner. For example, captive females which have not shown a phonoresponse but have been clasped by males will give the release call. This informs the male that the female has not attained the necessary physiological state for a successful amplexus to occur. *Xenopus* frequently breed in small dams in the wild, where chance collisions between frogs would be frequent. Males would therefore come into contact with females which had recently oviposited. The evolution of a well-developed female release call would have obvious advantages.

Russel (1960) believed that up till clasping the courtship is not specific, and that the release call of the female will determine whether amplexus is successful or not. This is contrary to the opinion expressed here, *viz.* that the call of the male and the phonoresponse of the female are the most important factors in conspecific recognition in *X. laevis*. This view emphasizing the importance of the mating call is supported by most workers, e.g. Salthe and Mecham (1974).

It was noted earlier that males overdosed with Pregnyl would over-respond sexually by omitting the mating call phase of the courtship. This underlines the need for caution in the use of hormones in studying sexual behaviour, and the interpretation of such data.

Müller and Scheer (1970) have published sonograms of the mating call of *X. l. laevis*. The locality of these frogs is given as Kenya. Vigny (1977) records only two species of *Xenopus* from Kenya, *X. borealis* and *muelleri*. The most northerly distribution record of *X. l. laevis* is from Zambia. (Poynton 1964; Vigny 1977). Müller and Scheer's (1970) sonogram of '*X. l. laevis*' does not correspond with those obtained in the present study, or of Vigny (1979). It shows no similarity to those given by Vigny (1979) for *X. borealis*, but does show some resemblance to that of *X. muelleri*.

Vigny (1979) notes that the second component of the call (S component) has fewer notes or an equal number of notes as compared to the first part of the call (R component). This was not found to be the case here, where 78% of frogs had more pulses in the S component than in the R component (n=32). Vigny (1979) noted a speeding up of the mating call and associated this with sexual frenzy of the male. The mating call, when given in isolation, was never noted to speed up in this study. It is only when a pair are in amplexus, and the female is unreceptive and resisting that the call of the male speeds up. This 'rapid calling' (Vigny 1979) does therefore not appear to relate to the increased sexual excitement of the male, but can be explained alternatively as an attempt by the male to remain in amplexus with an unresponsive female. Vigny (1979) noted that in the rapid calls of the male the S component of the call is always reduced 'from three to no notes'. Rapid calls of males in this study frequently showed more than three pulses, often as many as ten.

Only a small number of females tested gave a positive phonoresponse (5%). This result is not surprising since it is thought that females react to the call of the male for a very brief period (Schitz 1973), and that in mate choice experiments the percentage of responding females is frequently very low (Capranica, R. R. September 1979, pers. comm.) The percentage of responding females would be further reduced in laboratory situations dealing with hormonally stimulated animals, as some of the females may still be approaching the correct physiological condition for a phonoresponse, whereas others may have already ovulated.

Acknowledgements

The author expresses his gratitude to Dr Neville Passmore for his readily available technical assistance, constructive discussions and valuable criticism of the draft copies of this paper. Receipt of a CSIR bursary, Witwatersrand University senior bursary and the E.P. Bradlow scholarship are gratefully acknowledged.

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