DEVELOPMENT OF AUDITORY SYSTEM AND ACOUSTICALLY MEDIATED BEHAVIOUR IN ANURANS

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FROGS FEEL PHYSICAL JOY AND EXPRESS IT IN SONG

— MARY C. DICKERSON

Dedicated

To

My parents

Dr. Debjani Roy
Reader ,
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Mawlai, Shillong-793022.

CERTIFICATE

This is to certify that the thesis entitled " DEVELOPMENT OF AUDITORY SYSTEM AND ACOUSTICALLY MEDIATED BEHAVIOUR IN ANURANS " , submitted by Miss Bijoylakshmi Borah in fulfillment of the requirements for the degree of Doctor of Philosophy of North-Eastern Hill University , Shillong embodies the record of original research of work carried out by her under my supervision . She has been duly registered and the thesis submitted is worthy of being considered for the award of the Ph. D. degree .

This work has not been submitted for any other degree to any other university or institution .

Date : 20.7.98

Place : Shillong .

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Bijoylakshmi Borah
(Bijoylakshmi Borah)

Dated 20 7 98

Shillong

CONTENS

SYNOPSIS

INTRODUCTION

The progress from aquatic to terrestrial living was one of the most important events in the evolution of higher vertebrates. In the ontogeny of amphibians, the change from fish to tetrapod is faithfully repeated generation after generation. An aquatic larval period precedes the terrestrial period. Frogs and toads undergo extensive change and after metamorphosis approach the amniote type. Their locomotor and respiratory organs become entirely remodeled. After metamorphosis they develop new nutritional habits and accordingly the digestive system suffers radical change. In fact, the entire body becomes rebuilt, with the exception of the sex glands, which are little affected and attain maturity only after metamorphosis.

In view of the general, physiological and evolutionary importance of metamorphosis in the amphibians, it is surprising that it has never been investigated in a systematic way. One of the important organ which has not received due attention is the **EAR** or the **AUDITORY SYSTEM** during metamorphosis, though the morphological and functional change from underwater hearing in premetamorphic aquatic tadpoles to aerial hearing in adult terrestrial frogs accompanied by replacement of the larval accessory apparatus by entirely new tympanic organ of the adult are spectacular. Thus, the development of the amphibian ear and its sound perception system represent an astounding example of adaptation to changing environmental conditions.

It is with this view, the present work has been formulated for proper understanding of the morphological and physiological development of the auditory system as well as the development of acoustically mediated behaviour in *Limnonectes limnocharis*.

MORPHOLOGICAL AND FUNCTIONAL BACKGROUND

When vertebrates first ventured onto land, they had to adapt to terrestrial condition in various ways. This holds true also for hearing, because the properties of sound are quite different in air than in water. Fishes possess inner ear with otolith organs. Since water has approximately the same density as fish, sound waves in water run through the fish and vibrate its body. A fish with its inner ear thus vibrated. Airborne sound of normal intensity, however, will not bring the animal's head into vibration. Therefore, the air filled middle ear has been developed, the components of which such as the tympanum and the columella bridge the impedance mismatch between the airborne sound and the fluid filled inner ear.

These changes, that had occured during vertebrate transition to land, are repeated today in the development from the tadpole to frog. Tadpoles possess an inner ear but no middle ear. At later stage, lungs develop. The lung becomes connected to the inner ear through the round window. It has been argued, that this connection supports tadpole hearing similar to the way swimbladder support hearing in fish. The middle ear begins to develop at early metamorphosis, completion of development may take 2-12 months or more depending upon the geographic location and climate of the breeding area. The connection between the lung and inner ear ends at metamorphosis and the presumed input site for sound in the tadpole- the round window now becomes the release site of the inner ear for the sound wich comes in via the columella through the oval window. Even when the middle ear has developed fully in the young froglet, the size of the head is considerably smaller than in the adult frog. Since the frog ear acts as a pressure gradient receiver which reacts according to the pressure differance between the sound impinging from the outer side of the tympanum and the sound impinging on the typmanum's inner side via the the eustachian tube, thus the size of the head should also have a considerable impact on the hearing ability of the frog.

CURRENT STATUS

Most of the reports on amphibian auditory system and anuran acoustic communication has been from adult full grown frogs (Capranica 1976; Gerhardt 1982; Hetherington and Lombard 1982; Littlejohn 1977; Roy and Elepfandt 1993; Ryan 1986; Wells 1977; Wever 1973). The reason may be the following :

1. The biophysics of hearing in frogs is so different from that of mammals and also very complicated, that investigations have been concentrated for a long time on understanding the adult amphibian hearing.

2. The difference of underwater sound from the airborne sound necessitates a different experimental set up than for studies on airborne sound.

The few literature available for work on premetamorphic or early postmetamorphic stages are mostly on either morphological features of the ear of the transforming larva (Paterson 1949; Roy and Elepfandt 1990; Sedra and Michael 1959; Spaeti 1978; Weisz 1975; Witschi 1949) and that too, all these works are mostly on **Xenopus** or on kin recognition in tadpoles (Blaustein and O' Hara 1982; Blaustein, Bekoff and Daniels 1987; Blaustein and O' Hara 1988; Waldman and Adler 1979; Waldman 1981).

AIMS OF THE PROPOSED PLAN OF WORK

The aim of the proposed plan of work is to investigate the ontogeny of the auditory system, development of hearing and the behavioural responses exhibited to define sound stimuli. Amphibians provide interesting model for changes that may have occured during vertebrate evolution from water to land. So far however, amphibian hearing has been examined in adults only. Morphologically tadpoles possess an inner ear but the middle ear that is found in adult anurans develop not before metamorphosis. Hypotheses have been made about how tadpoles might hear, but experimental evidence that they do hear is lacking. Also, according to the present model of hearing in postmetamorphic anurans, hearing abilities should change with the size of the head. The project is aimed at testing hearing abilities of tadpoles and young froglets and by this, assessing the present model of hearing in *Limnonectes limnocharis*.

Thus the basic questions to be answered are :

1. At what stage during metamorphosis do the tadpoles or the young froglets begin to hear ?
2. What behavioural responses do these animals exhibit to define sound stimuli ?
3. What are the related morphological chnges in the sound conducting system ?

EXPERIMENTAL PROGRAMME

It will be essential to mention at this juncture why *Limnonectes limnocharis* has been choosen as the particular species to be studied :

1. In order to study the morphological details of the auditory structure, the normal table of development for this species will be needed. The normal table of development has already been studied (Roy and Khare 1978).
2. *Limnonectes limnocharis* colony has to be set up. For this the frogs have to induced bred, fertilized eggs have to be reared at their particular preferred temperature and finally the animals have to be fed with the food of their preference. Data on all these aspectes are already available (Khare, Kumar and Roy 1981; Roy and Khare 1979; Roy 1987; Roy 1990).

3. For experiments on acoustically mediated behaviour, a prior knowledge of the adult call pattern will be essential. This is already recorded and analysed (Roy and Elepfandt 1993).

A. RAISING AND MAINTAINING BREEDING COLONY UNDER NATURAL AND LABORATORY CONDITION

For any in depth study of neuroethology, it is of utmost importance to have a good animal facility. This would give a ready supply of animals needed for experimentation of any age, sex and weight. Continuous observation of the animals kept in the facility will also lead to a better " **feeling** " for the animals and their behaviour.

i. Colonies under natural conditions

A water reservior of 3 m x 3 m will be dug with 2 ft. depth having a slope. This will be surrounded by 2 m wide grass path. The entire area will be covered by wire netting 8 ft. high. Two 100 w lamps will be lit at night which will trap insects as food for the animals.

Initially the experimental species will be collected immediately after their hibernation period. Some animals will be left undisturbed to breed naturally, while others will be induced bred to obtain fertilized embryos.

ii. Colonies under labortaory conditions

Clean big aquariums with proper lighting, heating (if needed) and supply of aerated recycled water has to be set up. Initially a large variety of food will be tried, as the animals may show preference for a particular food item. This may vary according to the prey as well as the predator size. Once the preferred food item is known these food have to be reared under natural conditions. Extra care and experience will be needed to handle sick and weak animals for long period during behavioural testing in isolated conditions.

Time frame - 6 months.

B. MORPHOLOGICAL STUDY OF THE TRANSFORMING LARVAL AUDITORY SYSTEM DURING METAMORPHOSIS

The different larval stages after measuring their head trunk length, tail length and head width will be fixed either in mercuric chloride, trichloroacetic acid and formalin or in Zenker fixative. The former fixative will be used for tadpoles upto metamorphosis stage and the later for young metamorphosed and adult frogs. Immobilised, perfused animals will be fixed for 48 hours. After fixations the animals will be washed in 4% lithium sulphate and water for 24 hours respectively. Decalcification will be done in EDTA of pH 7.4 at 37^0 C. After decalcification the specimens will be washed once again in 4% lithium sulphate and water respectively.

Cryostat sections of the head region both in saggital and frontal plane will be prepared. Sections will be stained by trichrome stain for differential staining of the cartilaginous and calcified structures of the auditory system. This study will give the total histomorphological picture of the developing sound conducting apparatus.

Time frame - 7 to 12 months.

C. STUDY OF THE DEVELOPMENT OF ACOUSTICALLY MEDIATED BEHAVIOUR IN METAMORPHOSING TADPOLES

In view of the general physiology and evolutionary importance of metamorphosing frogs, it is surprising that this has never been investigated in a systematic manner. Hearing has been investigated in adult full grown frogs. Behavioural responses of the newly metamorphosed anurans is still poorly understood. Conclusive experiments are needed in order to elucidate the tadpole's ability to hear. Comparative morphological studies combined with the analysis of function will provide the data necessary for understanding the evolutionary significance of morphological features. The emphasis will be on the following questions :

a. At what stage during metamorphosis do tadpoles or young froglets begin to hear ?

b. What behavioural responses do these animals exhibit to define sound stimuli ?

c. What are the related morphological changes in the sound conducting apparatus ?

For answering these questions, extensive phonotaxis experiments will be conducted in acoustically isolated chambers.

D. WHETHER SPECIES SPECIFIC CALLS ARE LEARNED OR INNATE ?

This part of the work can only be started after the breeding colonies are successfully set up. Fertilized eggs will be obtained by induced breeding. 50 % of the fertilized eggs will be raised in acoustically isolated environment. The rest 50 % of the eggs will be raised along with the adult colony. The following will be observed and recorded :

a. When do the young metamorphosed froglets give the first call ?

b. Do the call parameters change as the animals grow old ?

c. What different types of calls do they emit - related to their behaviour pattern ?

d. Is there any differences between the calls emitted by the 2 groups - one raised in isolation and the other in colony ?

Time frame - 25 to 36 months.

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Chapter - 1

INTRODUCTION

The amphibian fauna of India is well known largely owing to the efforts of a number of works from mid nineteenth century. A few important workers are Stoliczka (1870), Boulenger (1882-1884; 1886-1888; 1890-1892; 1894; 1896-1897; 1904; 1908; 1918-1920), Thurston (1888), Fergusson (1904), Annandale (1907-1913; 1917a, b; 1918-1919), Allen (1910), Rao (1915; 1920; 1923; 1937), Smith (1917; 1927; 1929; 1935; 1940; 1953), Wall (1922), Hora (1923), Kampen (1923), Bhaduri (1929; 1933; 1944-1945; 1954-1956; 1980), Mc Cann (1932; 1934; 1938; 1940; 1945-1946), Parker (1934), Myers (1942a, b, c; 1968), Romer (1949), Abdulali (1954; 1954a, b; 1955; 1956; 1962), Acharji and Kripalani (1951), Kripalani (1961), Satyamurthy (1967), Murthy (1967-1968), Mohanty-Hejmadi (1974), Dutta (1985-1987; 1990a, b, c), Pillai (1973; 1976-1981; 1978-1980; 1981a, b;1986), Khan (1979), Mansukhani and Sarkar (1980-1981), Sarkar (1984-1985; 1990; 1993; 1993a, b).

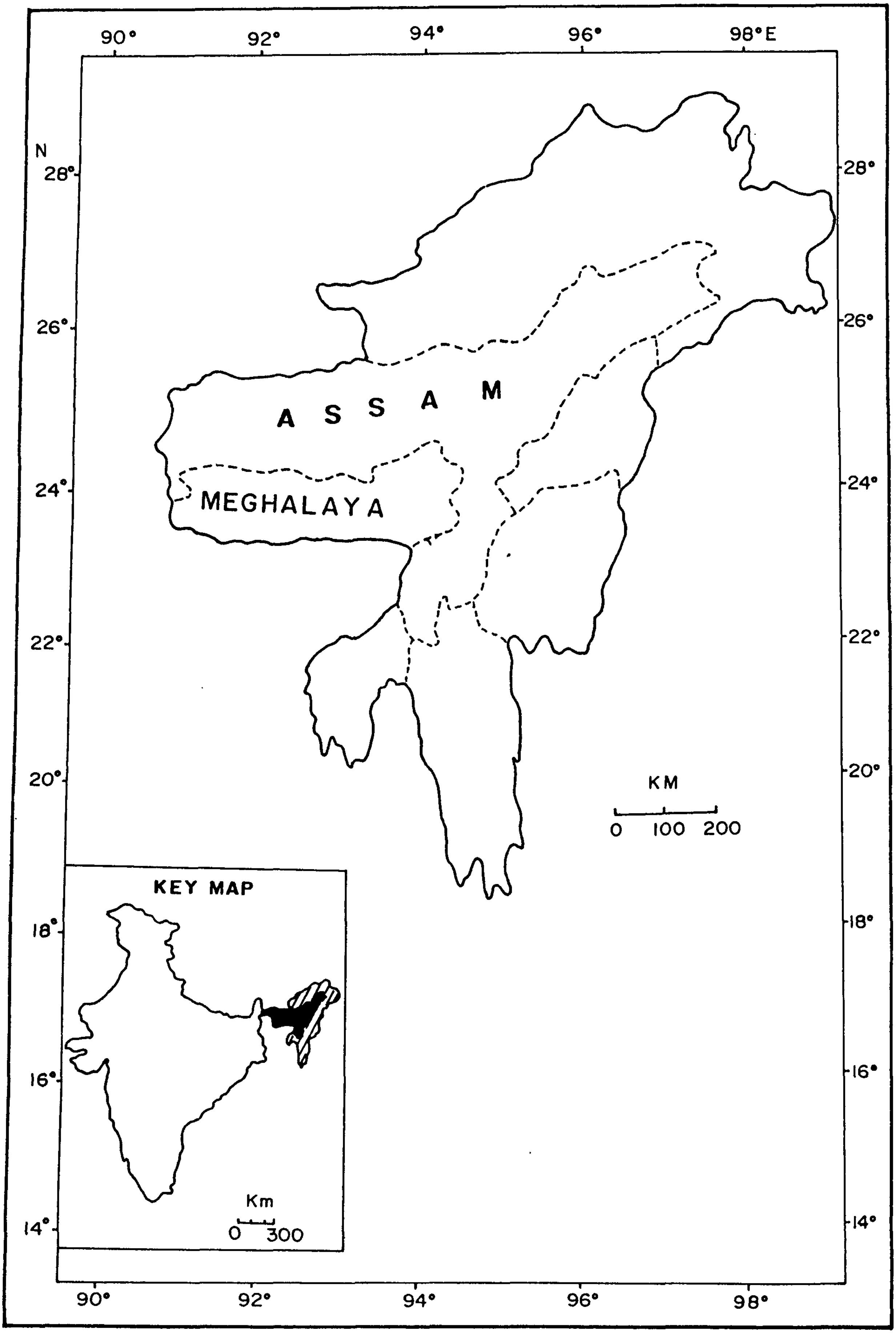
It is obvious looking at the above mentioned literature, that very little attention has been paid to study the amphibian fauna of northeast India. Among the important workers of this region are : Boulenger (1890-1892; 1894; 1896-1897; 1904; 1908; 1918-1920), Romer (1949), Roonwal and Kripalani (1961), Pillai and Chanda (1973; 1976-1977; 1979; 1981), Sahu and Khare (1983), Kiyasetuo and Khare (1986), Chanda and Ghosh (1988-1989), Chanda (1986; 1988-1991; 1994), Roy and Elepfandt (1993), Roy (1994), Roy, Borah and Sarma (1995), Roy, Sarma, Borah and Bannett (1998). Pillai and Chanda (1973) published an account of the distribution pattern of amphibia from Meghalaya, Assam, Arunachal Pradesh and Manipur from northeast India . Subsequently , Chanda (1986) made a comprehensive study of the amphibian fauna of northeast India on the basis of survey and collection work.

He identified the species based on their morphological characters. The colouration pattern of some amphibians have been described by him on the basis of the colouration of fixed specimen. This colouration is markedly different from that of the live specimens.

Anurans (frogs and toads) are abundant and occur in most habitats of northeast India due to its varied climatic conditions, vegetation and topography. Northeast India is mainly a mountainous terrain, largely of tertiary origin (Fig. 1). This part of India is remarkable for the luxuriant vegetation and humid tropical climate. It has three distinct physical features : (I) The Brahmaputra valley or the Assam valley, (II) The Surama Valley or the Barak valley and (III) a series of mountain and hill ranges, with the Himalayas in the north, the Khasi, Garo and Jaintia hills in the south; the Patkoi and Naga hills and the Manipur plateau in the east and the Lushai hills extending from south of Manipur to Burma as the Arakan Yoma. West Bengal, Bangladesh and Bhutan lie in the West of northeast India.

The Brahamaputra valley is an old alluvial plain stretching over an approximate area of 750 x 80 Km, with the Himalayas of Arunachal Pradesh and Bhutan in the north, the Meghalaya in the South and the Patkoi and Naga hills in the east. The main river is the Brahamaputra which rises from the Himalayas in the north. The Surama valley is a plain through which flows the river Surama, raising on the southern slopes of the mountains along the border of Naga hills. The Garo hills in the west rise sharply from the southern plain of Bangladesh. The Khasi hills in the east of the Garo hills, rise abruptly to an elevation of over 1200 m at a distance of 18 Km in the south, while in the north, rise from the Assam plain is gradual. The southern part along with the central one of the area is the Shillong plateau of Meghalaya. The Mikir hills are, however, a residue from the Meghalaya hill range projecting igure 1.

Map of Northeast India showing the two states - Assam and Meghalaya.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into the Brahmaputra valley.

It is well known that northeast India has highly tropical climate. As a whole the climate of this region is generally cool. It is difficult to determine the average annual temperature for the region as a whole. The annual rainfall is up to 250 cm or more, reaching the highest rainfall scale in the world, as Mawsynram of Meghalaya. There are four main seasons in this region - Winter, Summer, Monsoon and Autumn. The monsoon generally withdraws from northeast India between the last week of September to first week of October.

Northeast India offers a wide variety of vegetation type due to its varied climatic conditions. The luxuriant vegetation of this region may be divided into three types: (I) Tropical, (II) Temperate and (III) Alpine vegetation.

Out of the world's 4522 amphibian species (WWF, India 1994) 207 are from India. The northeast alone contributes 56 species under 29 genera and 8 families (BCPP CAMP Report on Amphibian, 1997). As is evident from the literature on the survey records from northeast India, this region still remains unexplored due to its difficult terrain, indicating there may be many more species yet to be recorded. Until recently the method followed for amphibian systematic was by field survey, where frogs were captured randomly from their natural habitat, killed and preserved . The identification of the preserved specimen were based on morphological characters. These preserved specimens lost their natural colouration due to the action of the fixative, which at times led to erroneous description of their colour pattern. This method of random collection also led to severe population depletion and habitat destruction.

Realizing the severe population depletion of the amphibians worldwide, it was felt that the present method of survey was harmful. Since the last two decades, the role of species specific anuran mating call has been emphasized (Ryan 1985; Zelick et. al. 1991). The species specific calls form one of the major identification criteria for amphibian systematics (Roy and Elepfandt 1993; Roy 1994; Sinsch and Schneider 1996; Roy et.al. 1998). The bioacoustic analysis of species specific call may result in identifying new unrecorded species (Dubois 1975; Schneider et. al. 1979; Roy and Elepfandt 1993; Sinsch and Schneider 1996). The present status of literature on northeast amphibian species clearly indicated that a detailed survey based both on morphological characters and bioacoustic analysis was necessary. It was with this objective in mind, a survey was done to identify the commonly available species in Assam and Meghalaya.

MATERIALS AND METHODS

For the survey work several visits were made to different places in Assam and Meghalaya. Collections were done from different habitats such as marshes, pools, streams, rivers, banks, sandy soil, cultivated fields, grasslands, on trees, shaded mountains etc. During survey - habitat, vegetation, air and water temperature, humidity and rainfall were noted. Morphological measurement (snout to vent length for males) , diagnostic features(Fig. 2 a, b, c, d and e) , photographs and call recordings were done . The specimens were then either released back in their natural habitat or carried, to be kept, in the froggery adjacent to the institute under seminatural condition.

OBSERVATIONS

The following amphibian species were found from Assam and Meghalaya during the survey :

Figure 2.

Different body parts :

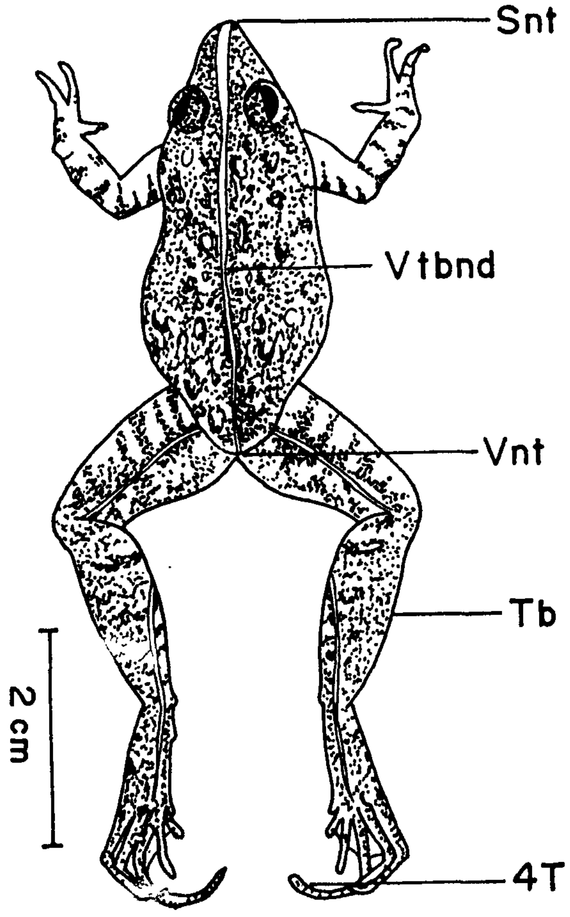
a. Dorsal view of frog showing vertebral band and snout vent length.

b. Lateral view of the head region of frog showing skin fold above tympanum.

c. Text figure showing tibio-tarsal articulation in relation to the position of the eye and snout.

Abbreviations -

Snt - Snout	Fd - Skin fold
Vtbnd - Vertebral band	Ey - Eye
Vnt - Vent	Tym - Tympanum
Tb - Tibia	Nst - Nostril
4th - 4th toe	Tbtra - Tibio-tarsal articulation
H. limb - Hind limb	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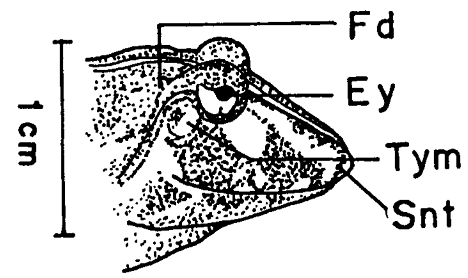
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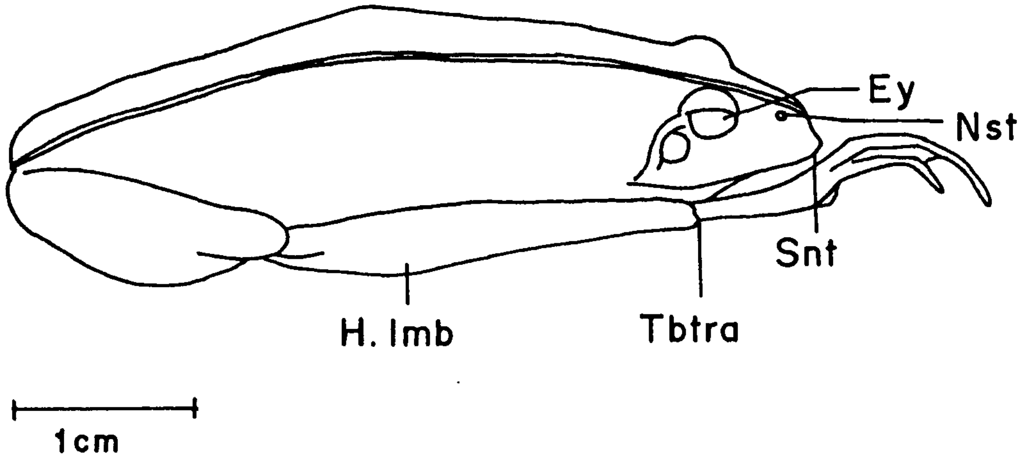

Ey
Nst
Snt
H. lmb
Tbtra
1cm

c.

Figure 2.

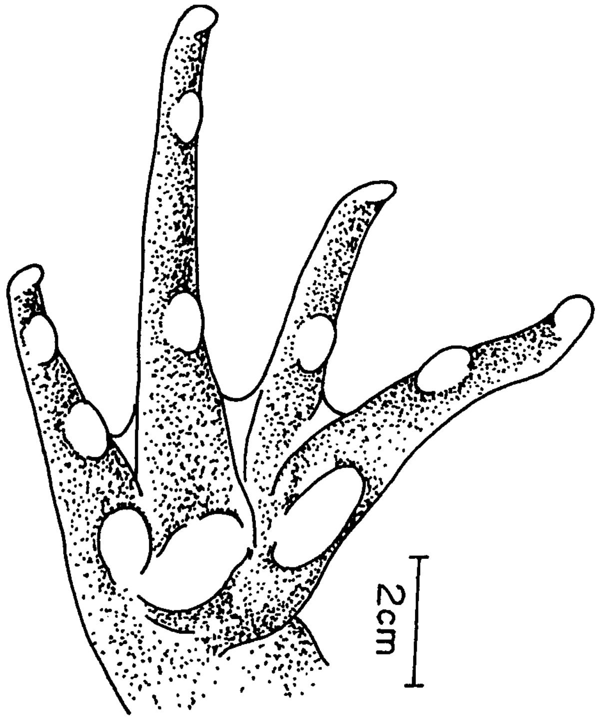
Different body parts :

d. Pattern of fingers in the forelimb of frog

e. Pattern of toes in the hindlimb of frog

Abbreviation -

Wb - Webb.



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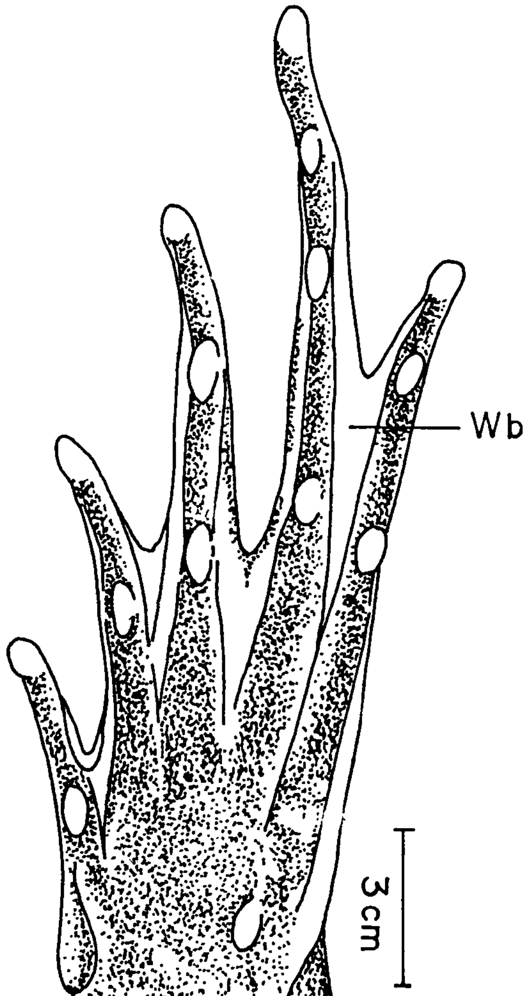


e.

1.*Limnonectes limnocharis* - Plate 1 (i) A

Habit and habitat

It is commonly known as cricket frog. It is generally found in shallow marshes , damp grasslands near canals and ditches and in flooded fields during the rainy season. Before and during the breeding period they hide among vegetation and under stones. They are good jumpers. When disturbed escape into dense vegetation and water. They do not remain in deep water for long but swim back immediately to the bank. They are quite agile.

Measurement

Snout to vent length 47.5 - 60.0 mm .

Diagnostic features

Small to medium sized frogs. Head is almost as long as broad. Snout is somewhat pointed and projects beyond the mouth. Nostril is nearer to the tip of the snout than the eye. Eardrum is distinct, nearly 2/3 the diameter of the eye. Fingers are without webs. The first finger is longer than the second. Tips of the fingers and toes are swollen but not disc like. Joints between the segments of the fingers and toes are prominent. Toes are half webbed with three segments of the fourth toe free. A distinct oval inner pedal tubercle is present. Outer pedal tubercle is less prominent. The joint between the shank and the ankle reaches between the eardrum and nostril, when the hindlimb is held parallel to the body. Some short and interrupted longitudinal glandular folds are present on the back. A prominent skin fold run from behind the eye to the shoulder. Belly is smooth.

During monsoon nights, the males call somewhat like the chirping of a cricket.

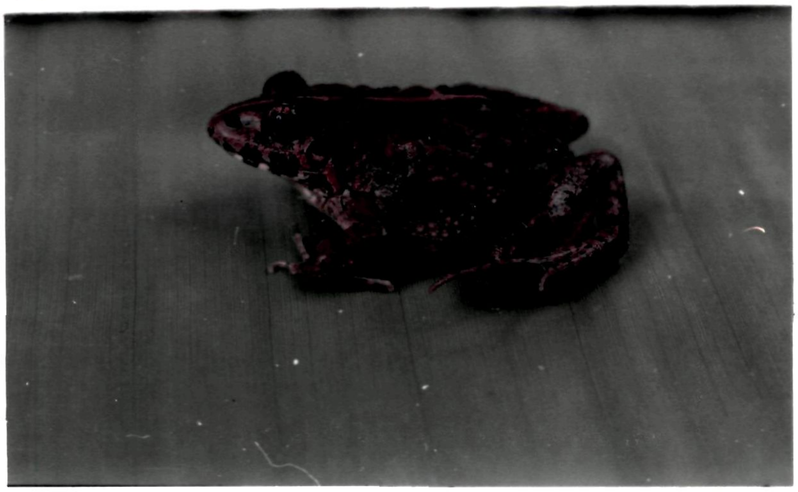
Plate 1 (i).

A - ***Limnonectes limnocharis*** **.**

B - ***Microhyla ornata*** **.**

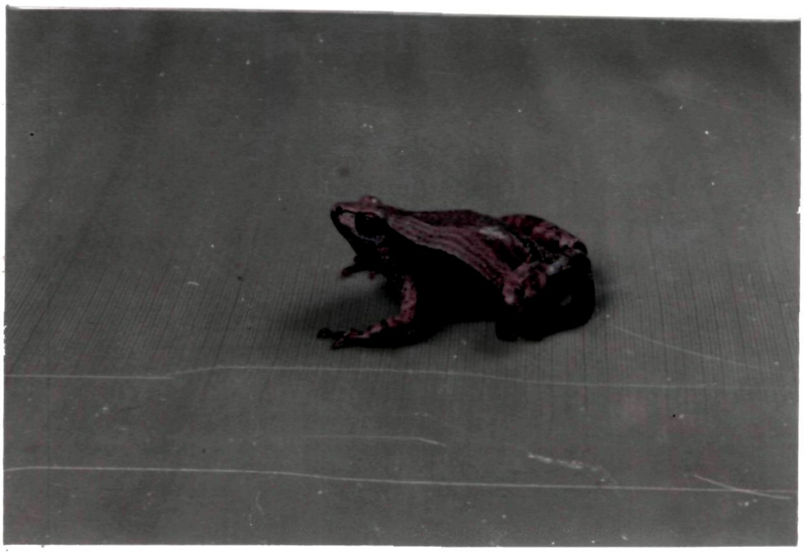
PLATE-1(i)

A



2cm

B



1cm

Colouration

Dorsal surface gray with black spots. A wide whitish median line. Ventral surface white or creamish. Throat gray, sometimes black granulate.

Distribution

All over India, including northeastern states, Sri Lanka ,China, Malaysia, Indonesia, Philippines.

In northeast India, *L. limnocharis* exhibits certain intraspecific variations specially in colour pattern in reference to the dorsal vertebral line which may be totally non-existent or sometimes very broad and prominent.

2. *Microhyla ornata* - Plate 1 (i) B

Habit and habitat

The species is common. They are terrestrial and nocturnal. They are normally found in the grasses and bushes, on the banks of ponds and tanks. They hide under stones and debris. They occur throughout the year in forest and agricultural lands but their numbers and calls become prominent after a heavy shower during the monsoons. They are very agile in comparison to their size.

Measurement

Snout to vent length 14.0-25.0 mm .

Diagnostic features

Small sized, slender, active frogs with bulging eyes. Head is broader than long. Snout is somewhat pointed and projects beyond the narrow mouth. Nostril is nearer to the tip of the snout than the eye. Eardrum is indistinct. Fingers are slender and do not bear webs. The first finger is shorter than second. Tips of the fingers are flattened . Rudiment of webs present between the toes. The tips are blunt. Two small but distinct and oval (inner and outer) pedal tubercles are present. The joint between the shank and the ankle reaches near the eye when hindlimb is held parallel to the body. Skin is smooth.

Despite their small size, the males have a loud and distinct call.

Colouration

The upper side is light brown with distinct dark brown diamond shaped marking over the back. A dark streak extends along the sides from behind the eye to the shoulder. Limbs are with dark cross bars. The belly is dull white.

Distribution

India, Sri Lanka, Pakistan, Nepal, Bangladesh, Burma, Thailand, South China, Malay Peninsula, Taiwan and Japan.

3. *Limnonectes khasiana*

Habit and habitat

It is plentiful in rice fields and gardens.

Measurement

Snout to vent length 35.0 - 36.0 mm .

Diagnostic features

Dorsally uniform reddish brown in colour, spotted with brown on lateral sides. Thighs faintly barred. Chin, thorax and legs spotted brown. Dorsal skin smooth. No trace of tubercle. Ventral surface almost smooth. Head short, broader than long. Snout short and rounded. Eyes large and prominent. Tympanum small, covered by skin, faintly visible. Fingers free. Three small subarticular tubercle present on the palm. Hindlimbs are short.

Tibia is slightly shorter than thigh. Inner metatarsal tubercle shovel shaped and laterally compressed. Outer metatarsal tubercle is absent.

The calls are loud and given in rapid succession.

Colouration

A tiny reddish brown species. Dorsal side with brownish triangular dark patches.

Distribution

India (Meghalaya).

4. *Hyla annectens* - Plate 1 (ii) C

Habit and habitat

Commonly known as garden frog. Mainly found in potato fields and in the garden climbing from one tree to another. Out of the 260 valid species of this genus from the world, it is the only species found in northeast India.

Measurement

Snout to vent length 23.0 - 48.0 mm .

Diagnostic features

Dark green to light green colour on the dorsum. A light brownish streak from eyes to nostrils. A black lateral streak present upto groin , often terminating in two black spots of different sizes with interconnections. Ventral surface of thigh yellowish . A few black spots , arranged more or less in a line present on ventral surface of femur and tibia. Dorsal skin smooth. A strong tuberculated fold extend from the eyes to shoulder, over the tympanum. Ventral surface smooth.

The calls are loud and noisy.

Plate 1 (ii).

C - *Hyla annectens* .

D - *Polypedates maculatus* .

PLATE-1 (ii)

C

2 cm

D

2 cm

Colouration

Dorsal surface dark green with a light brown s treak from eyes to nostrils. A black lateral streak present upto groin often terminating into two black spots of different sizes with interconnections. Ventral surface of thigh yellowish. A few black spots arranged more or less in line present on the ventral surface of the femur and tibia.

Distribution

India (Meghalaya, Assam, Nagaland), Burma, Thailand, China and Vietnam.

5. *Polypedates maculatus* - Plate 1(ii) D

Habit and habitat

This frogs is found in paddy fields and marshy grasslands. It is also found among potato field and bean plantation. The calling males conceal themselves under potato leaves or other vegetation.

Measurement

Snout to vent length 35.0 - 48.0 mm

Diagnostic features

Medium sized, slim, narrow waisted tree frogs with slender elongated limbs and goggling eyes. Head is broader than long. Snout is somewhat pointed and projects a little beyond the mouth. Nostril is nearer to the tip of the snout than the eye. Eardrum is distinct, nearly equal to the diameter of the eye. Fingers are rudimentary webs. The first finger is nearly equal to the second. Joints between the segments of the fingers and toes are very prominent. Toes are almost half webbed with two segments of fourth toe free. Tips of the fingers and toes are dilated into flattened spherical or horse-shoe shaped adhesive disc. It has a smooth skin and granular belly and underside of the thighs. On its back are a pair of distinct elevation, which is observed only when it is at rest.

The male has a loud voice syllabilized as TAK-TAK- .

Colouration

Dorsally yellowish brown to dark brown. Limbs with brown and white cross bars of irregular patterns. Thighs and throat light brown to yellowish. The chin and throat are with dark large oval spots.

Distribution

India (Meghalaya, West Bengal, South India except Punjab, Haryana and Rajasthan), Sri Lanka, Nepal and Bangladesh.

6. *Euphlyctis cyanophlyctis* - Plate 1 (iii) E

Habit and habitat

These frogs are aquatic in nature. They are found in pools, muddy swamps and canals. These frogs can remain in water for long without coming on to the land and spend most of their time floating motionless with eyes and tip of the snout above water. When alarmed they skitter across the water surface for several feet before diving and hiding on to bottom.

Measurement

Snout to vent length 44.5 - 66.5 mm .

Diagnostic features

Medium sized pond frogs. Head is broader than long. Snout is rounded. Nostril is equidistant from the tip of the snout and eyes. Eardrum is distinct, nearly equal to the diameter of the eye. Fingers are without webs. The first finger is equal to the second. Tips of

Plate 1 (iii).

E - *Euphlyctis cyanophlyctis* .

F - *Rana alticola* .

PLATE-1(iii)

E

2 cm

F

2 cm

the fingers are pointed. Joints between the segments of the fingers and toes are less prominent. Toes are fully webbed. Tips of the toes are swollen and rounded. A prominent toe like inner pedal tubercle is present. Outer pedal tubercle is absent. The joints between the shank and the ankle reaches in between the eardrum and the nostril when the hindlimb is parallel to the body. Small warts are present on the upper side. Glandular longitudinal folds are absent on the back but a prominent skin fold runs from behind the eye to the shoulder. Belly is smooth with a single row of porous warts on each flank.

They are highly vocal during the monsoons. During monsoon after sunset, a single note low-pitch call is heard which soon spreads into a chorus as the night progresses. The males sit at the side of the water bodies and croak continuously by inflating the paired bluish white vocal sacs which protrude out through slits on the floor of the mouth.

Colouration

The colour of the body is either grey, greyish-brown or greyish-black with darker rounded spots on the back and stripes on the limbs. The belly is white with black spots.

Distribution

All over India including northeastern states, Pakistan, Afghanistan, South Arabia and Sri Lanka.

7. ***Rana alticola*** - Plate 1 (iii) F

Habit and habitat

It is found in ponds, ditches, beels and low lying areas abundant in aquatic vegetation.

Measurement

Snout to vent length 41.5 - 54.0 mm .

Diagnostic features

Dorsally yellowish to light brown. A feebly prominent, narrow, glandular dorsolateral fold running from posterior region of eyes above the tympanum to hip. Ventrally almost white. Skin smooth. A feeble prominent glandular lateral fold present. Another glandular fold running from the posterior region of tympanum to shoulder. Head longer than broad, depressed. Snout slightly longer than eyes, obtusely pointed, projecting slightly beyond mouth. Forelimbs short. Fingers are long, slender, free with horse-shoe shaped disc, separating upper from lower surface. First finger slightly longer than second, third longest, much longer than snout. Hindlimbs are long, slender, tibiotarsal articulation reaching beyond tip of snout. Tibia five to six times as long as broad. Toes having small disc like fingers, but slightly broader than the later, entirely webbed. A small indistinct outer metatarsal tubercle present.

The calls are repeated very rapidly.

Colouration

Dorsally yellowish to light brown. Two distinct glandular dorsolateral folds running from the anterior region of the body to the posterior region ending near the groin.

Distribution

India (Assam, Meghalaya, Tripura, West Bengal, Orissa, Andaman), Sri Lanka, Nepal, China, Japan, Indonesia, Malaysia.

8. *Polypedates leucomystax* - Plate 1 (iv) G

Habit and habitat

The species is quite common . Found in agricultural land and thin forest . They are nocturnal and arboreal , hiding during the day time by sitting immovably on branches of bushes and especially among bamboo-shoots by tucking in all the limbs under the body. This species have been mostly collected from Assam . They are found on creepers entwining bamboo fencing or tall grass near the vicinity of water.

Measurement

Snout to vent length 52.0 - 57.0 mm .

Diagnostic features

Large sized tree frogs with elongated limbs and bulging eyes. Head is broader than long. Snout is not pointed and projects slightly beyond the mouth . Eardrum is distinct nearly equal to the diameter of the eye. Fingers are without webs. The first finger is nearly equal to the second. Toes are almost fully webbed with a single segment of the fourth toe free. Tips of the fingers and toes are dilated into horse-shoe shaped adhesive disc. It has a smooth skin and granular belly.

The call consists of separate deep croaks.

Colouration

The colouration is variable. Upper parts light brown with few dark parallel stripes and specks but under parts whitish. There are dark markings on the body especially on the sides of the head.

Distribution

India (Assam , Meghalaya , West Bengal , South India , Manipur , Arunachal Pradesh), Nepal, South China, Bangladesh, Java, Japan and Thailand.

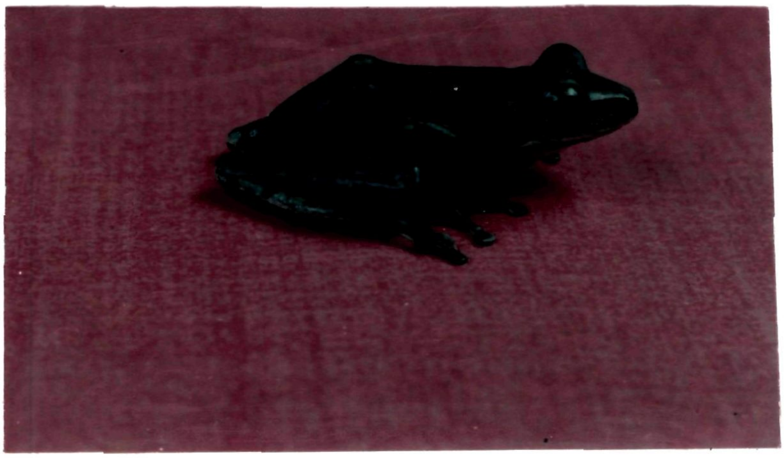
Plate 1 (iv).

G - *Polypedates leucomystax* .

H - *Rana livida* .

PLATE-1 (iv)

G



2cm

H

2cm

9. ***Rana livida*** - Plate 1 (iv) H

Habit and habitat

Mostly found in beels, ditches and ponds having aquatic vegetation. They are mostly found during summer.

Measurement

Snout to vent length 47.5 - 78.2 mm .

Diagnostic features

Dorsally gray , sometimes a row of large black spots present on the head which are smaller towards interorbital region in adult . A prominent white streak present on the upper jaw. Tympanum brown white with clear whitish circular rim. Limbs with brownish crossbands. Ventrally light to dark brown. Skin smooth , flanks and posterior parts of thighs granulated. A glandular fold originating from the posterior corner of eyes to shoulder , followed by a glandule . Head is as long as broad, much depressed. Snout slightly pointed, projecting little beyond mouth. Forelimbs are short . Fingers free , moderately large with very faint dermal border. Tips of fingers with prominent discs. Hindlimbs are very long. Tibiotarsal articulation reaching beyond tip of snout. Toes are fully webbed. Subarticular tubercle moderately large and prominent.

Colouration

Dorsally gray, sometimes a row of large black spots present on the head. A prominent white streak present on the upper jaw. Ventrally light to dark brown.

Distribution

India (Assam, Meghalaya, Manipur and West Bengal).

10. ***Amolops formosus*** - Plate 1 (v) I

Habit and habitat

These frogs are found both by the sides of streams as well as undwelled forests caves.

Measurement

Snout to vent length 53.0 - 75.0 mm .

Diagnostic features

Dorsally bright green with blackish spots on the head and body. Limbs with cross bands. Posterior part of thigh marbled with black. Lower parts and web between the toes whitish to brown. Dorsal skin smooth. A glandular fold present above the tympanum. Belly granulated. Males with internal vocal sacs. During breeding season a thick pad found on the inner side of the first finger.

The call is long a continuous trill.

Colouration

Green with black patches on dorsal surface. Ventrally creamish with black patches mostly concentrated in lower jaw and throat region.

Distribution

India (Assam, Meghalaya), Nepal.

11. ***Bufo melanostictus*** - Plate 1 (v) J

Habit and habitat

This species is found throughout the year. They are found mainly on land, under stones and damp places.

Plate 1 (v).

I - ***Amolops formosus*** **.**

J - ***Bufo melanostictus*** **.**

PLATE-1 (v)

I

2cm

J

2cm

Measurements

Snout to vent length 75.0 - 80.0 mm .

Diagnostic features

Dorsally brown or yellowish brown. Sometimes with black spots. Tips of warts and ridges of head usually deep brown to black. Ventrally yellowish, more or less spotted with brown. Dorsal skin rough with more or less prominent spiny warts and tubercles of various sizes. Ventral surface smooth.

After a heavy shower, the males congregate and start croaking from the banks of ponds, ditches and stagnant rain water pools.

Colouration

Brown to yellowish brown. Dorsally with black spots. Tips of warts and ridges of head usually deep brown to black. Ventrally creamish at times with light brown spots.

Distribution

India, Burma, Sri Lanka, China, Malaysia, Indonesia, Philippines.

12. *Hoplobatrachus tigerinus* - Plate 1 (vi) K

Habit and habitat

It is commonly known as tiger frog. Found near water in weed choked ponds, ditches, tanks and marshes. Although these frogs are strong jumpers and swimmers, it is very easy to catch them since they keep sitting for hours together. They are solitary creatures, aggregating only in the breeding season.

Measurement

Snout to vent length 100.0 - 200.0 mm .

Diagnostic features

Large frogs with smooth skin marked with large irregular blackish spots which may be disposed in longitudinal series on the back or forming two or three cross bands. A dark band running anteriorly from eye to snout over the nostril. Ventrally white, without spots or few spots on the throat. Dorsal skin covered with longitudinal folds. A strong glandular fold extends from eye to shoulder. Head is as long as broad. Snout pointed, projecting much beyond lower jaw. Nostrils nearer to tip of snout than to eye . Tympanum is very distinct , 2/3 of eye. Forelimbs are much shorter than hindlimbs. Finger free, long with rounded tips. Subarticular tubercles small but very prominent. Hindlimbs are very long, toes long, entirely webbed with prominent rounded tips. Prominent dermal fold present on inner side of fifth toe. Outer metatarsal tubercle absent.

In the non-breeding season, they are difficult to locate but with the appearance of the rainy season their highly resonant call ' QUANK QUANK ' can be heard throughout the night.

Colouration

Dorsally yellowish to olive green with darker leopard like spots. Younger frogs paler mostly grass green in colour bearing a yellowish lateral band behind eyes which are absent in the adult. Ventrally white, without spots or few spots on the throat.

Distribution

All over India including northeastern states, Nepal, China, Burma, Sri Lanka, Bangladesh, Thailand, Taiwan.

Plate 1 (vi).

K - *Hoplobatrachus tigerinus* .

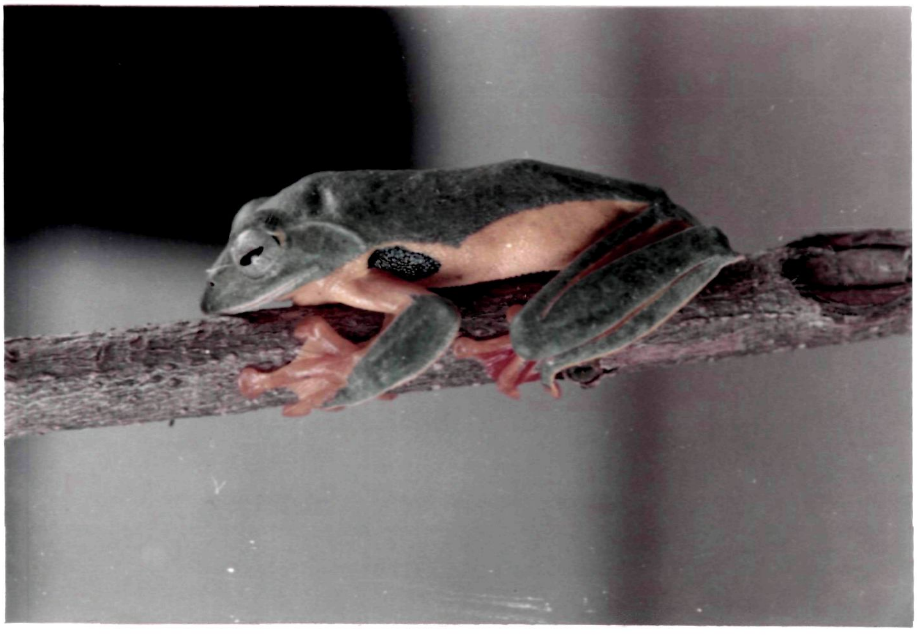
L - *Rhacophorus bipunctatus* .

PLATE-1 (vi)

K

2cm

L



2 cm

13. ***Rhacophorus bipunctatus*** - Plate 1 (vi) L

Habit and habitat

They are found on creepers and tall grass near the vicinity of water.

Measurement

Snout to vent length 45.0 -50.0 mm .

Diagnostic features

Colour variable. Dorsally green to olive, leaf green in live. Ventrally yellowish. The characteristic black spots are very prominent on flanks. The numbers of spots are variable, sometimes two large spots occur on the right side with three smaller spots on the left. The anterior spot is larger than others. Dorsal skin smooth . Belly, lateral sides of body and thighs fully granulated. Head broader than long, slightly depressed. Snout obtusely pointed. Nostrils equidistant from eyes and tip of snout. Tympanum is distinct, 2/3 of eyes. Forelimbs are moderately long. First finger is shorter than second. Third finger is longest, longer than snout. All fingers webbed to discs except the first. Hindlimbs are long. Tibiotarsal articulation reaching posterior corner of eye. Toes with large discs and with circum-marginal groove, fully and broadly webbed. Outer metatarsal completely separated by web. Subarticular tubercles small and prominent.

Colouration

Dorsum is green to pale green. Ventrally yellowish. Toes are broadly webbed with red colour web. Black spots on each side are very prominent.

Distribution

India (Meghalaya, Assam, Arunachal Pradesh, Manipur, Nagaland, Tripura), Indonesia.

DISCUSSION

The anuran fauna of northeast India is rich when compared to the anuran fauna of India, constituting nearly 1/3 of the total species known from the Indian subcontinent. It is significant that representatives from all the 8 families of the anurans found in India have been reported from northeast India. Out of the 43 genera so far recorded from India, 29 genera occur in northeast (BCPP CAMP Report on Amphibia, 1997). Dense tropical rain forest, high precipitation, varying altitudinal gradients, abundance of food species and relatively less eco-disturbances have possibly contributed to the richness and diversity of anuran fauna of this region.

The present study has been undertaken with a view to present an up-to-date account of some commonly available species of anuran, which represent different ecological conditions. Quite a number of species of the genus *Rana* have localized distribution. *L. limnocharis* and *E. cyanophlyctis* which are the most abundant species throughout northeast India, have been reported to be distributed not only in India but throughout southeast Asia (Fig. 3 and 4). The colour pattern of *L. limnocharis* shows considerable variations specially with regards to the dorsal vertebral line extending from snout to vent which is sometimes very prominent and broad and in some cases totally absent.

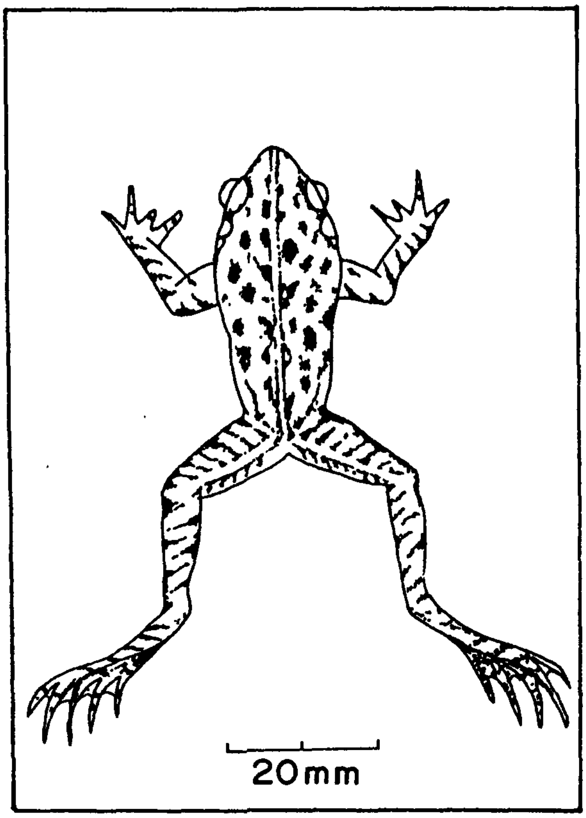
The anuran fauna of northeast India appears to be more closely related to the anuran fauna of north and south India. A considerable number of northeast Indian anuran species (16%) have been recorded from south and north India specially from the hilly region of Tamil Nadu (Annamallai hills), Karnataka (Silent Valley), Kerala (Wynad), Himachal Pradesh, Jammu and Kashmir (Rao 1937 ; Satyamurthy 1967 ; Murthy 1968 ; Pillai 1978 , 1980 ;

Figure 3.

a. ***Limnonectes limnocharis*** **(to scale).**

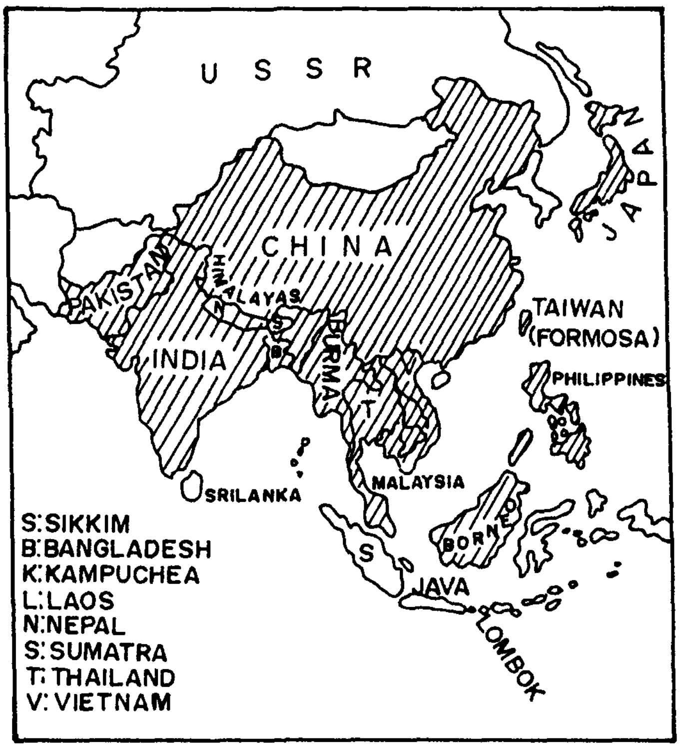
b. Distribution map of ***Limnonectes limnocharis*****.**

a.



LIMNONECTES LIMNOCHARIS

b.



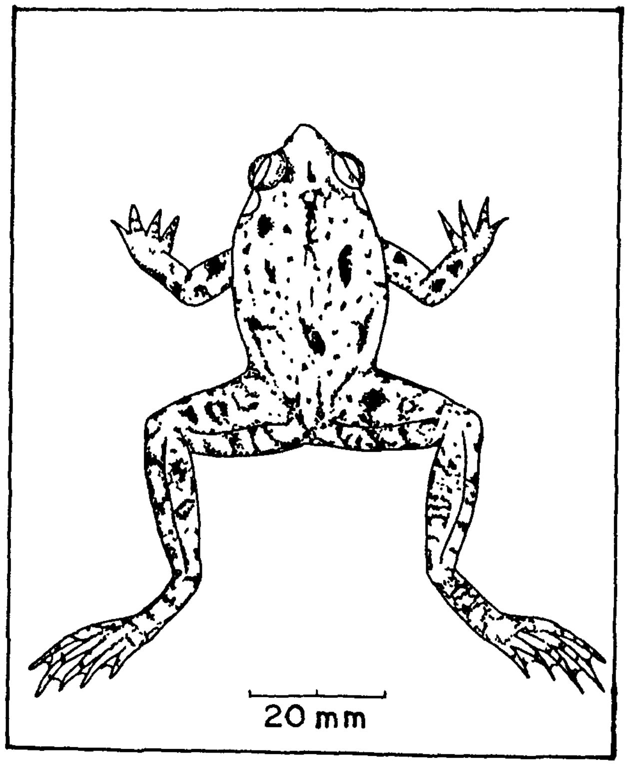
DISTRIBUTION MAP

Figure 4.

a. *Euphlyctis cyanophlyctis* (to scale).

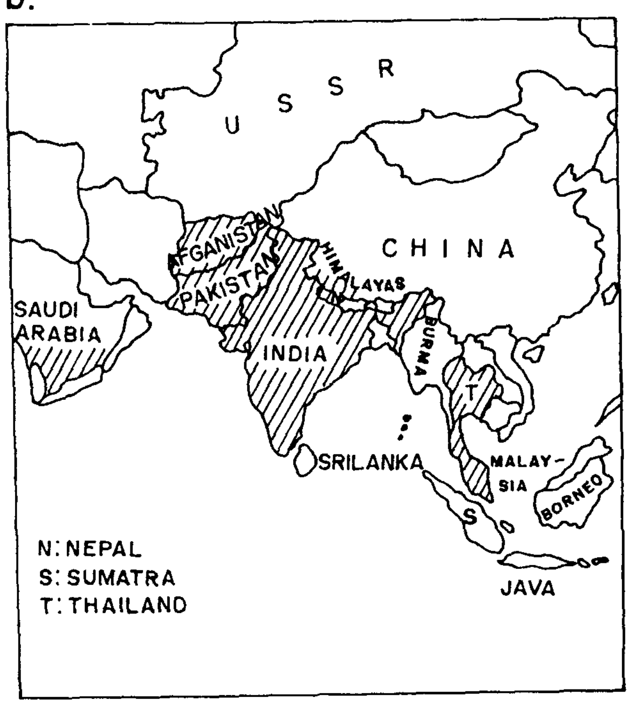
b. Distribution map of Euphlyctis cyanophlyctis .

a.



EUPHLYCTIS CYANOPHLYCTIS

b.



DISTRIBUTION MAP

Dubois 1980).

The northeast Indian anuran fauna also shows close relation with the Burmese anuran fauna and as many as 14 genera and 18 species from northeast India have been known to occur in Burma (Bourret 1942). The similarities in ecological conditions between northeast India and Burma perhaps have contributed towards close affinity of anuran fauna between the two regions.

The vertebrate fauna of India is generally well known. Yet new taxa continue to be discovered and more amphibian species await discovery. It is clear that more exploration is necessary in order to obtain an objective evaluation of India's true biodiversity which applies to the amphibian fauna as well. Unless the pace of exploration is accelerated especially given the rate of habitat loss, it is conceivable that atleast some of these may vanish even before they are discovered.

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Chapter - 2

INTRODUCTION

Field trips were conducted for surveying the anuran amphibians during early spring (March to May) for 2 consecutive years i. e. 1994 and 1995. Field work began daily at 1600 hours and continued for 3 hours. The survey and collection data (Table 1) showed that the numbers of frogs observed were much less in comparison to the manhours spent on survey. Moreover, the number of the females in general was much less than the males. The reason for lesser number of frogs (Deuti and Goswami 1995) could be indirectly attributed to :

(a) The direct effect of human activities like disturbance in the habitat and breeding grounds; draining of swamps and marshes ; filling up of wetlands ; felling of natural forests for agricultural purpose.

(b) Pollutions due to pesticides, detergents and toxic industrial effluents.

(c) Illegal frog hunting.

(d) Unscientific methods of collection.

All these factors are responsible for the declining amphibian population not only in India but worldwide. It was therefore, essential to have a good amphibian facility both for academic as well as conservation values. The facility generated has a ready supply of animals of any age, sex and weight. Handling the animals on daily basis for their maintenance provided ' better feel ' for their biology.

Besides the present study had a major component of behavioural biology. In order to study the behavioural pattern handling of animal on a daily basis was of added advantage to get to know their behaviours in relation to different physiological conditions and environmental effects.

Thus, it was with this cause and effect the animal maintenance facility to rear and maintain the frogs under semi-natural condition was built up.

A. MAINTENANCE UNDER SEMI-NATURAL AND LABORATORY CONDITION

i. Semi-natural pond adjacent to the institute

A pond measuring $10' \times 10' \times 2'$ built adjacent to the institute to rear frogs under semi-natural condition. Surrounding the pond is a footpath covered with grass. The total area of the frog enclosure together with the footpath is ($18' \times 16'$) and the height being $7'$. The entire area is protected by a small wire netting to prevent the frogs from escaping and also being eaten by the predators. Quarter of the roof of the pond is covered by galvanized sheet and the rest by wire netting. Two 100 W electric bulb lit at night. The insects get attracted to the illumination at night and fall inside the pond. The frogs get to prey upon these insects (Plate 1).

The pond has an inclination at one side with an water outlet. This outlet helps in removing the dirty water and cleaning the pond from time to time. The pond is filled with rain water.

For rearing of tadpoles and frogs in the pond some important criteria were followed. They were kept in a sufficient volume of water.

ii. Aquariums under laboratory condition

It is a challenging job to rear the frogs the whole year in laboratory. Yet, it was done providing sufficient amount of food and some special arrangement to keep the temperature optimum for the frogs.

ıte 1.

Frog enclosure adjacent to the Institute.

Table 1. Number of species collected and observed during the survey.

Species	Collection locality	Male						Female					
		Weight (gm)			SVL (cm)			Weight (gm)			SVL(cm)		
		Range	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range	Mean	SD
Limnonectes limnocharis	Guwahati (Male-25; Female-10)	4.0- 6.8	5.30	0.95	5.0- 5.2	4.30	0.73	6.8- 8.2	7.45	0.47	4.8- 6.0	5.43	0.46
	Nalbari (Male-12; Female-6)	4.5- 6.0	5.38	0.46	3.4- 5.2	4.12	0.56	5.8- 7.3	6.60	0.55	4.2- 5.0	4.76	0.33
	Byrnihat (Male-20; Female-8)	2.0- 5.2	3.70	0.84	2.7- 5.0	3.80	0.79	2.5- 6.0	4.90	0.98	2.5- 5.0	4.00	0.78
	Nongpoh (Male-12; Female-7)	2.8- 7.0	5.12	1.43	2.7- 5.0	3.78	0.82	6.0- 8.8	7.33	0.94	3.2- 5.1	4.11	0.76
	Shillong (Male-18; Female-8)	3.0- 6.5	5.04	0.88	3.0- 5.0	3.86	0.74	6.0- 7.2	6.56	0.54	3.8- 5.1	4.55	0.45
Microhyla ornata	Guwahati (Male-2)	2.0- 2.1	2.05	0.05	2.2- 2.5	2.40	0.15						
Limnonectes khasiana	Pariong (Male-4)	2.4- 3.0	2.70	0.22	3.4- 4.0	3.60	0.23						
Hyla annectens	Pariong (Male-9; Female-5)	4.0- 5.0	4.30	0.40	3.5- 4.2	3.80	0.24	5.0- 7.0	5.60	0.80	4.0- 6.2	4.90	0.74
Polypedates maculatus	Shillong (Male-10; Female-5)	3.8- 6.0	4.50	0.64	4.0- 4.8	4.50	0.23	6.0- 8.0	7.30	0.72	4.5- 5.0	4.80	0.64
Euphlyctis cyanophlyctis	Guwahati (Male-22; Female-9)	4.5- 7.6	5.99	0.74	3.0- 5.0	4.08	0.54	7.0- 9.5	8.33	7.41	4.5- 6.5	5.48	0.58
	Nalbari (Male-10; Female-7)	5.5- 7.5	6.51	0.68	3.8- 5.0	4.36	0.42	7.5- 8.5	8.03	0.28	4.5- 6.0	5.12	0.46
	Byrnihat (Male-15; Female-10)	4.3- 5.8	5.03	0.50	4.0- 5.5	4.67	0.48	5.0- 7.0	6.23	0.62	4.0- 5.5	4.82	0.45
	Nongpoh (Male-10; Female-6)	5.0- 7.0	5.74	0.69	4.0- 5.5	4.77	0.49	5.8- 7.5	6.52	0.58	3.9- 5.0	4.45	0.39
	Shillong (Male-10; Female-6)	5.5- 7.0	5.81	0.44	3.5- 5.0	4.13	0.41	6.5- 7.8	7.17	0.41	4.0- 5.0	4.43	0.38
Rana alticola	Guwahati (Male-10; Female-5)	6.5- 7.5	6.95	0.32	4.5- 5.3	4.96	0.25	7.0- 8.5	7.70	0.51	4.5- 5.5	5.02	0.33
Polypedates leucomystax	Guwahati (Male-20; Female-11)	4.5- 7.0	5.51	0.96	4.5- 6.0	5.37	0.39	9.0- 18.0	13.46	3.55	6.0- 8.5	7.37	1.12
Rana livida	Nalbari (Male-10; Female-5)	19.0- 23.5	20.40	1.70	6.5- 7.5	6.90	0.36	24.0- 28.0	25.80	1.80	7.0- 8.0	7.50	0.05
Rhacophorus bipunctatus	Shillong (Male-5; Female-2)	3.8- 4.2	4.00	0.13	4.0- 4.8	4.40	0.26	4.5- 5.0	4.80	0.25	4.8- 4.9	4.90	0.05
Amolops formosus	Pariong (Male-10; Female-5)	12.0- 30.0	22.30	5.20	5.0- 7.5	6.01	1.14	25.0- 40.0	33.00	5.10	7.0-10.0	7.90	1.24
Bufo melanostictus	Guwahati (Male-10; Female-6)	8.0- 14.5	19.80	10.96	6.0- 8.0	6.90	0.96	50.0-100.0	73.30	18.18	8.0- 9.5	8.70	0.52
Hoplobatrachus tigerinus	Guwahati (Male-10; Female-2)	105.0- 350.0	275.00	65.88	8.5- 17.0	12.75	2.44	110.0-450.0	350.00	139.82	12.5-20.0	17.63	3.07

PLATE-1

Aquariums were fabricated in 3 sizes : (75 x 45 x 45 cm), (75 x 30 x 30 cm) and (50 x 20 x 20 cm) to rear frogs, froglets and tadpoles of different sizes and number in the laboratory. Aquarium was filled up by pond water upto one and half inch and placed some stones as their sitting place. Some aquatic plants were placed inside the aquarium by which a natural environment was created. The water of the aquarium was changed twice a week. There were 13 species collected from different localities of Meghalaya and Assam. The animals were fed regularly in the breeding season. Earthworm was the main food item and termite was also supplied as a food for tadpole, froglet and adult frog. For arboreal frogs e.g. *Polypedates leucomystax, Hyla annectens, Rhacophorus bipunctatus* and *Polypedates maculatus* branches of trees were placed so that they climb up.

During winter temperature decreases. The air temperature of Shillong drops to 2^0-3^0 C during the winter month. The winter temperature is unsuitable for the maintenance of the frogs under laboratory condition. Room heaters were used to increase the temperature of the laboratory. The temperature is maintained around 12^0 C.

The tadpoles are totally aquatic, whereas the froglets after metamorphosis leave their aquatic habitat and become amphibious. To raise the colony from fertilized eggs to adult under laboratory condition, the inner decor of the aquariums should resemble the natural surroundings of the totally aquatic limbless tadpoles, tadpoles with limbs, young metamorphosing froglets and the froglets after they have completed metamorphosis. Accordingly two types of decors were used for the aquarium, which are as follows :

a. Aquarium for tadpole

Substrate : Peat moss layers covered with find sand or gravel.

Plants : *Polygonum sp. , Amannia, Limnophila, Lycopodium, Dicranopteris, Hypericum , Euriocaulon* and *Sphenomeris.*

Decoration : Hiding places near strategic plant arrangements, such as rock grottos or tangles of ariel roots.

Changes of water in the aquarium are of great value for normal development. In the first 2 days after fertilization, the water is changed twice a days and then until the transition to active feeding once a day and later once in 2 days. The tadpoles do not require constant aeration, but water plants such as - *Polygonum sp. , Amannia, Limnophila, Lycopodium, Dicranopteris, Hypericum , Euriocaulon* and *Sphenomeris* are useful in aquarium for oxygenation (Fig. 1A).

For the rearing of tadpoles and frogs in the aquarium some important criteria were followed. They were kept in a sufficient volume of water, such as 100 tadpoles in 5000 ml of water. We followed this volume of water for normal development, because if their density is higher the development of different individuals proceeds asynchronously and with a general delay. In this volume of water the tadpoles can be kept until metamorphosis having no delay in the process.

Tap water should be dechlorinated in open vessels for not less than 2 days. Insufficient dechlorinated water can induce rapid mass death of animals. Changes of water in the pond are of great value for normal development.

Egg clutches of *Limnonectes limnocharis* , *Polypedates leucomystax* ,*Polypedates maculatus* and *Rhacophorus bipunctatus* were seen in early spring. The fertilized eggs developed well till the froglet stage, with 95 % survival. But maximum mortality was seen

Figure 1 A.

Aquarium for tadpole .

Figure 1 B.

Aquarium for froglet .

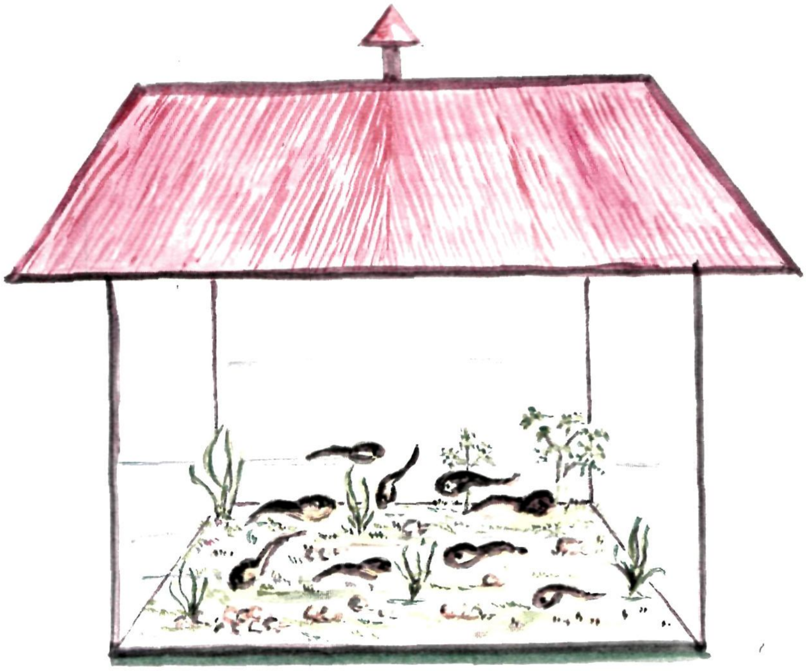

Fig. 1A. Aquarium for tadpole

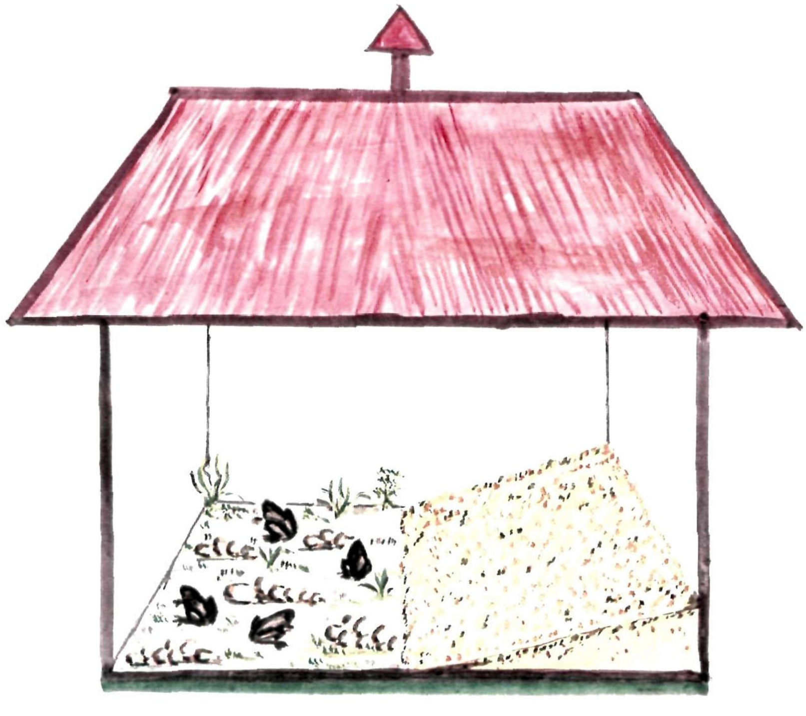

Fig. 1B. Aquarium for metamorphosed froglet.

after metamorphosis, when there was a dietary change.

b. Aquarium for metamorphosed froglet

During metamorphosis when the froglets tend to leave the water and come up on land, very little water is filled in the aquarium. Then aquariums are inclined, so that a portion of the aquarium remains dry. The bottom is covered with filter paper so that froglets does not slip too much coming out of the water. Small pebbles and branches of houseplants are placed on the water free part of the bottom. The aquariums are covered with nets so that the metamorphosed frogs cannot jump out. During this period water is changed daily (Fig. 1B).

The metamorphosed frogs are placed in the aquarium in small groups. A small amount of cotton covered with filter paper and well wetted with water is placed on the bottom, together with small pebbles and branches. The aquariums are covered by a net, cleaned everyday and cotton and filter paper are changed.

B. FEEDING

From the beginning of active feeding, the tadpoles are fed with the albumin of hard boiled eggs. Within 2 - 3 days the egg is supplemented with plant food such as boiled cabbage and spinach. With in a few days the spinach or cabbage is replaced by small earthworms. Earthworm is given to tadpoles in the morning, and in the afternoon its residues are removed from the aquarium and the water is changed.

During the period of metamorphosis the tadpoles do not feed. After metamorphosis the froglets are fed with drosophila flies and earthworm (Deltcaff and Vassetzky 1991).

C. FOOD FOR ANIMALS

It was felt that in order to have a proper animal facility both under semi-natural and laboratory condition, the food supply should be independent from any market supply or field collection of food animals. It was therefore felt necessary to raise and maintain a culture of preferred food animals. Earthworm was found to be the most preferred food item.

Keeping and breeding important food animals

1. Earthworm

We used the following process :

a. Black soil 4 - 5 kg.

b. Vegetable wastes.

c. Used tea leaves.

These mixture are essential for earthworm culture. In an aquarium we mixed all these above materials and mixed water (a little). Sometimes we kept big sized earthworm there. After two to two and half months we got enough earthworms.

2. Fruitflies (*Drosophila melanogaster*)

Container

250 - 1000ml beakers or jars covered with fine gauge.

Maintenance

Covered bottom of container with about 2 cm thick layer of food medium. Climbing facilities were provided by placing sticks of filter paper, wood shearings or similar material on top of medium at 25^0 C, RH 80 - 90 %.

Food

Food medium made of milk and or similar cereal, mixed with a few grains of dry yeast and some liquid multivitamin sprinkle. Some fungus inhibitor was provided over the mixture in order to prevent fungal growth.

Availability

Initially rotten fruits e. g. banana were kept on an open surface. This attracted the fruit flies. When enough number of fruit flies were obtained they were used to start the culture.

D. CARE FOR SICK ANIMALS

Fungal infections cured by immersing animals in 0.002 % wt / vol $KMnO_4$ aqueous solution for 30 minutes daily. The animals sometime perished due to leg paralysis as they cannot swim to the surface for air and as a result get suffocated. However, if the water level is lowered in time, the animals are saved. They donot eat for a long time, some times up to several weeks.

Unlike Nieuwkoop and Faber (1967) recommendation running water avoided, since it has been found to cause a condition resembling red leg disease, which results in the death of the animals. Aquariums emptied and refilled with clean water twice a week. To prevent fungal diseases, we add a few milliliters of salt solution with every change of water.

The temperature for keeping animals are 16 -20^0 C and they can live normally at 10 -12^0 C . The animals are kept under artificial light which is switched on during the night.

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Chapter - 3

INTRODUCTION

In order to study the development of acoustically mediated behaviour in young froglets, we have first got to know the call structure of the conspecific adults. We also know that there exists a direct or inverse correlation between the frequency and body length. Therefore, before taking up the study on acoustically mediated behaviour in the froglets, it was necessary to study the morphological characters and call pattern of the adults.

The morphological measurements of anurans till early 1980's played an important role in amphibian systematics and taxonomy. The existing faunal record of 56 anuran species from northeast India is based on morphological characters (BCPP CAMP Report on Amphibian 1997). Recent works from both within the country (Roy and Elepfandt 1993 ; Roy, Borah and Sarma 1995 ; Roy, Sarma, Borah and Bannette. 1998) and outside (Ryan 1985, 1986 ; Schneider and Sinsch 1992 ; Schneider et. al. 1993 ; Sinsch and Schneider 1996) have demonstrated that anuran mating call constitute an important character for species identification more distinctive than morphological characters. Frogs that once have been considered as belonging to one species based on their morphological characters turn out to belong to different species on the basis of their mating call.

Rana limnocharis (Limnonectes limnocharis) in Nepal has been subdivided into four species on the basis of their mating call (Dubois 1975). Advertisement call differences from morphologically alike species *Rana limnocharis (Limnonectes limnocharis)* from northeast India has also been reported by Roy and Elepfandt (1993), Sarma (1997) suggesting the so called *Limnonectes limnocharis* from northeast India is not one species but a composition of several species of similar morphological appearance. Roy et al. (1998) described the call pattern in terms of temporal and spectral characters, absolute measurements and biometrics ratios for 10 amphibian species from northeast India.

A relationship between size and frequency components of the advertisement call has been reported in studies of anuran communities (Duellman and Pyles 1983). This correlationship index has also been used by (Zimmerman 1983) to seek differences between conspecifics in different habitats.Thus, it is clear that there are differences in the call pattern between the conspecifics elsewhere as well, other than Nepal and northeast India.

Measurement of the morphological character and call analysis of the so called *Rana ridibunda* at the Gallikos river near Thessaloniki, showed considerable differences from the recorded morphological characters and call patterns of *Rana ridibunda* . It was for this reason this frog was classified as a new water frog from Greece *Rana epeirotica* (Schneider et al. 1984).

In recent years there have been many bioacoustic studies of the anurans in Greece which have made a major contribution to the animal taxonomy. The method has succeeded even with species that present special problems because of their reclusive habits or the low intensity of their calls for instance the toad *Bufo bufo spinosus* (Sofianidou and Schneider 1985) and the frog *Rana dalmatina* (Sofianidou and Kyriakopoulou Sklavounou 1983 ; Schneider et al. 1988).

Having seen the importance of morphological characters and call pattern analysis in amphibian systematics and taxonomy and to correlate the adult call pattern with the young froglets a survey was taken up during the breeding season of the anuran amphibians for two consecutive years i. e. 1994 and 1995 in northeast India. Absolute morphological measurements and call recordings were done for the species found during the survey.

MATERIALS AND METHODS

Survey, call recordings and collections were carried out in Assam and Meghalaya during the breeding period from May to August on 2 consecutive years, 1994 and 1995. Field recordings and collections were made daily from around 6 p.m. sometimes until midnight.

The calls were recorded on a professional SONY WM 6 DC cassette recorder with an unidirectional AKG C451EB shotgun condenser microphone held approximately 40 - 60 cm away from the calling frog. The calls were stored on Maxell XLII cassette tapes. Sound pressure level was measured by playing back isolated calls on a Philips double cassette player DR 920 with playback volume fixed at volume 3 and the CYGNET 2021 sound pressure level meter held approximately 1 m away from the sound source.

Recorded acoustic stimuli were digitized via a Microsoft analogue- to- digital interface board on to an IBM PC and stored on diskettes. Oscillograms shown as waveform display of amplitude versus time trace; sonogram as the frequency versus time trace with amplitude and mean spectra showing the maximum energy concentration at a particular frequency band were prepared with a computerized Fast Fourier Transformation (FFT) system after passing through band pass filters.

Measurements of the following 15 absolute morphological measurements were recorded from atleast 5 or more individuals each for males and females separately from each of the 10 species studied. All morphological measurements were accurate to 0.1mm.

A. MORPHOLOGICAL CHARACTERS

1. Body length : Snout to vent length (SVL).

2. Body width : Measured at the widest part across the abdomen.

3. Head width (min.) : At the tip of the snout parallel to the nostril.

4. Head width (max.) : Measured at the widest part across the eyes.

5. Snout length : Perpendicular distance from below the nostril to the tip of the mouth.

6. Nostril diameter : Measured across the long axis of the nostril.

7. Internarial distance : Distance between inner margin of the nostril bordering flaps .

8. Eye diameter : Transverse distance from below the nostril to the tip of the mouth.

9. Interocular distance : Transverse distance between inner bases of the circum-orbital plaques.

10. Hindlimb length : Vent to tip of the fifth toe.

11. Tibia length : Medial measurement of the outer ventral surface of the digit.

12. 4th toe length : Measurement of the outer ventral surface of the digit.

13. Total forelimb length : Origin of the limb to the tip of the first finger.

14. Lower forelimb length : Outer angle of the elbow to the tip of the first finger.

15. First finger length : Base to the tip of the 1st finger.

B. CALL CHARACTERS

1. Call duration (sec) : Duration from the beginning of a call to its end.

2. Call period (sec) : Duration from the beginning of a call to the beginning of the next call.

3. Pulse number : The number of individual components of each call (FFT length - 256 : Overlap - 50 % ; Window - Hamming).

4. Dominant frequency (kHz) : The frquency with maximum intensity.

5. Frequency domain (kHz) : The range of frequencies that differ by less than 10 dB intesity from the dominant frequency.

After noting down the morphological measurements and recording the calls, few specimens from each species were preserved in 8 % formaldehyde for photography and drawing and the rest were released in their natural habitat.

OBSERVATIONS

15 absolute measurements (Table 1) and temporal and spectral data after acoustic analysis of the mating calls (Table 2) of 10 anuran species from northeast India have been studied. Figs. 1A-10A represents the line drawings for the different frog species (to scale); Figs.1B-10B show the mean spectra of the mating calls and Figs.1C-10C the corresponding sonagram (FFT length : 256 ; Overlap : 50 % ; Window : Hamming) Fig. 11 shows the waveform representation or oscillogram of a single call for each of these 10 species.

Limnonectes limnocharis (Figs. 1ABC and 11; Tables 1and 2)

1. Habit and habitat

Mostly known as the cricket frog. It is generally found in shallow marshes, damp grasslands near canals and ditches and in flooded field during the raining season. Before and during the breeding period they hide themselves among vegetation and under stones. They are good jumpers, when disturbed escape into dense vegetation and water. They do not remain in deep water for long but swim back immediately to the bank.

2. Colouration

Dorsal surface grey with black spots. A wide whitish median line. Ventral surface

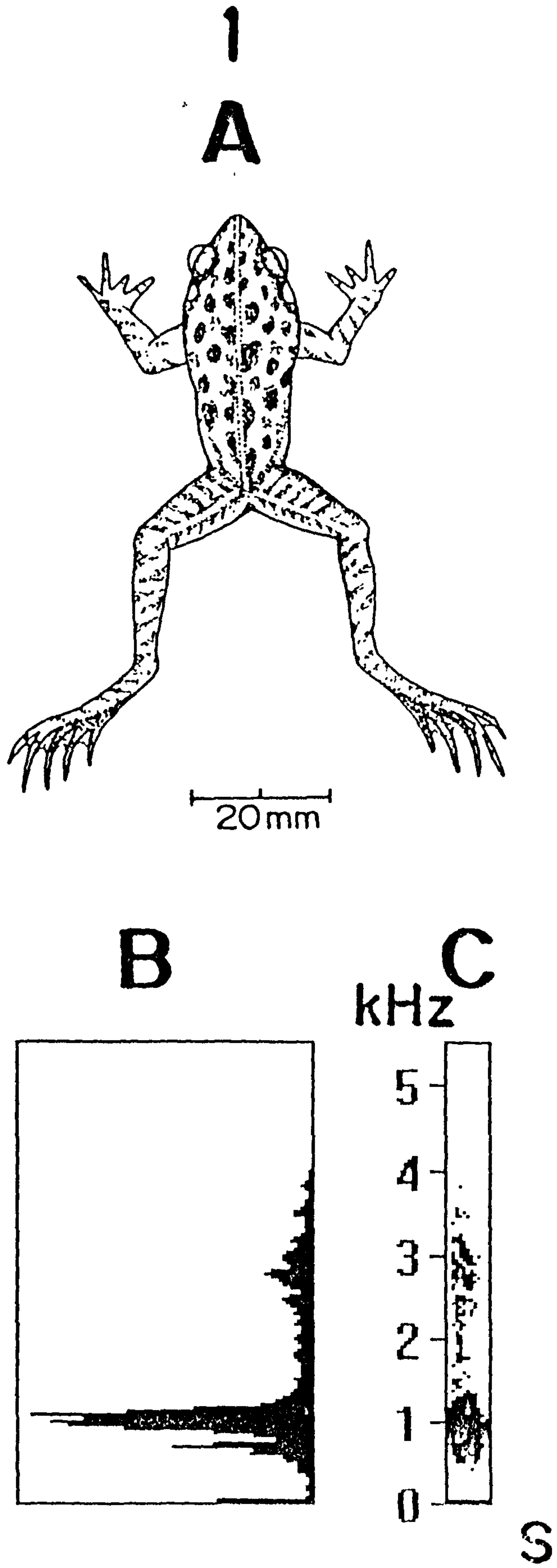
Figure 1.

A. Line diagram of *Limnonectes limnocharis* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)



LIMNONECTES LIMNOCHARIS

Figure 11.

Oscillograms shown as waveform display of amplitude versus time trace for a single mating call of -

A. Limnonectes limnocharis; B. Limnonectes khasiana;

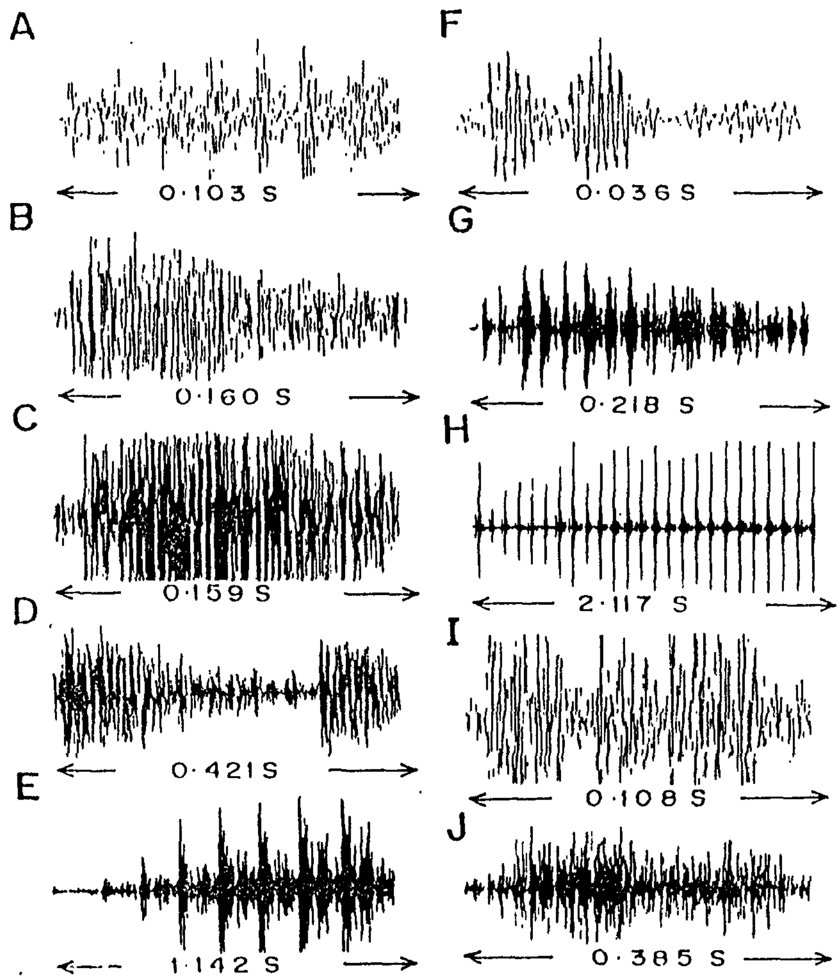
C. Hyla annectens ; D. Polypedates maculatus ;

E. Euphlyctis cyanophlyctis ; F. Rana alticola ;

G. Polypedates leucomystax; H. Amolops formosus;

I. Bufo melanostictus; J. Hoplobatrachus tigerinus.

(FFT length-256; Overlap- 50%; Windows- Hamming)



A
0·103 S
F
0·036 S
B
0·160 S
G
0·218 S
C
0·159 S
H
2·117 S
D
0·421 S
I
0·108 S
E
1·142 S
J
0·385 S

Table 1. Morphometric measurements of 10 amphibians from Assam and Meghalaya.

Characters	*Limnonectes limnocharis*		*Limnonectes khasiana*	*Hyla annectens*	
	Male(10)	**Female(10)**	**Male(10)**	**Male(7)**	**Female(5)**
Body length	4.11±0.36	4.75±0.36	3.50±0.05	3.82±0.17	4.83±0.23
Body width	2.11±0.37	2.50±0.21	2.40±0.10	1.37±0.04	1.93±0.18
Head width (min)	0.96±0.10	1.12±0.04	0.65±0.05	0.67±0.04	0.80±0.14
Head width (max)	1.50±0.13	1.70±0.07	1.80±0.10	1.12±0.08	1.50±0.00
Snout length	0.70±0.08	0.75±0.05	0.45±0.50	0.52±0.08	0.70±0.00
Eye diameter	0.48±0.05	0.50±0.07	0.45±0.50	0.47±0.12	0.56±0.04
Interocular distance	0.33±0.11	0.40±0.07	0.35±0.50	0.50±0.00	0.73±0.04
Nostril diameter	0.20±0.00	0.20±0.00	0.10±0.00	0.17±0.04	0.20±0.00
Internarial distance	0.46±0.04	0.40±0.04	0.70±0.00	0.30±0.00	0.40±0.00
Hindlimb length	6.48±0.57	7.62±0.45	5.30±0.10	5.70±0.12	7.83±0.47
Tibia length	1.75±0.21	2.17±0.12	2.40±0.10	1.70±0.12	2.20±0.16
4th toe length	1.82±0.10	2.37±0.23	1.20±0.10	1.70±0.12	2.30±0.14
Total forelimb length	1.51±0.30	2.12±0.12	2.10±0.10	2.72±0.08	3.40±0.14
Lower forelimb length	1.33±0.21	1.42±0.14	1.30±0.10	2.03±0.16	2.53±0.23
1st finger length	0.61±0.09	0.70±0.07	0.45±0.05	0.45±0.05	0.53±0.04

Continued

Polypedates maculatus		*Euphlyctis cyanophlyctis*		*Rana alticola*	
Male (10)	**Female (6)**	**Male (10)**	**Female (10)**	**Male (7)**	**Female (6)**
4.14±0.17	4.56±0.27	3.90±0.32	3.40±0.66	5.10±0.08	5.45±0.05
1.80±0.08	1.80±0.10	1.60±0.04	1.40±0.32	1.33±0.12	1.65±0.05
0.96±0.08	1.02±0.04	0.90±0.18	0.70±0.18	0.96±0.09	1.15±0.05
1.50±0.06	1.58±0.11	1.30±0.00	1.10±0.19	1.46±0.16	1.75±0.05
0.68±0.04	0.76±0.08	0.63±0.04	0.58±0.09	0.86±0.09	0.08±0.00
0.56±0.04	0.61±0.04	0.53±0.04	0.47±0.08	0.66±0.04	0.65±0.05
0.52±0.07	0.55±0.07	0.43±0.04	0.35±0.07	0.50±0.00	0.45±0.05
0.12±0.04	0.10±0.00	0.23±0.04	0.22±0.04	0.20±0.00	0.20±0.00
0.30±0.00	0.36±0.08	0.33±0.04	0.32±0.04	0.50±0.08	0.55±0.05
6.52±0.21	7.12±0.69	5.80±0.28	5.10±0.81	7.30±0.07	7.60±0.10
2.12±0.16	2.34±0.24	1.70±0.04	1.50±0.21	2.20±0.08	2.45±0.05
1.92±0.07	1.98±0.21	1.90±0.09	1.70±0.35	2.06±0.09	2.25±0.05
2.80±0.10	2.92±0.27	1.80±0.04	1.60±0.28	2.90±0.21	2.65±0.05
2.02±0.29	2.16±0.28	1.40±0.00	1.00±0.27	1.76±0.16	1.65±0.05
0.06±0.04	1.90±0.24	0.76±0.09	0.60±0.15	0.65±0.05	0.75±0.05

Continued

Polypedates leucomystax		*Amolops formosus*		***Bufo melanostictus***	***Hoplobatruchus tigerinus***
Male(10)	**Female(10)**	**Male(10)**	**Female(10)**	**Male (10)**	**Male (10)**
5.40±0.18	8.40±0.26	4.43±0.13	8.20±1.03	7.80±0.20	14.33±4.18
1.60±0.22	2.63±0.16	2.20±0.08	3.58±0.36	3.90±0.08	5.16±0.84
1.20±0.11	1.80±0.04	1.20±0.08	1.82±0.09	1.60±0.12	3.60±0.14
1.90±0.09	2.70±0.04	1.80±0.08	2.60±0.42	2.90±0.08	4.40±0.29
0.97±0.08	1.30±0.04	0.86±0.04	1.18±0.16	1.10±0.05	1.90±0.12
0.68±0.03	0.80±0.00	0.86±0.04	0.81±0.39	0.10±0.05	1.70±0.08
0.78±0.11	1.10±0.00	0.46±0.04	0.76±0.12	0.65±0.05	1.10±0.04
0.10±0.00	0.16±0.02	0.20±0.00	0.22±0.04	0.30±0.00	0.23±0.04
0.41±0.03	0.70±0.00	0.56±0.04	0.88±0.21	0.50±0.00	0.63±0.04
8.35±0.16	11.90±0.32	8.66±0.13	13.74±1.33	9.90±0.30	20.70±2.56
2.77±0.26	3.76±0.20	3.04±0.04	4.66±0.22	2.60±0.12	5.40±0.28
2.28±0.21	2.93±0.30	2.32±0.09	3.74±0.17	2.70±0.12	5.30±0.49
3.45±0.04	5.23±0.20	3.16±0.04	4.84±0.67	5.00±0.05	6.60±0.41
2.40±0.15	3.80±0.14	2.32±0.09	3.70±0.20	3.00±0.05	4.50±0.20
0.68±0.06	1.16±0.24	0.86±0.04	1.36±0.17	0.65±0.05	1.20±0.08

Table 2. Acoustic analysis of the mating calls of 10 amphibian species.

Species (Male; n =20)	Call duration (Sec)	Call period (Sec)	Pulse number	Lower frequency (kHz)	Higher frequency (kHz)	Dominant frequency (kHz)	SPL (dB)
Limnonectes limnocharis	x 0.11 n 0.01	x 0.25 n 0.03	x 56 n 4.2	x 0.35 n 0.06	x 4.40 n 0.30	x 1.08 n 0.06	x 68.10 n 3.17
Limnonectes khasiana	x 0.15 n 0.01	x 0.22 n 0.02	x many n -	x 0.35 n 0.04	x 4.20 n 0.02	x 0.67 n 0.03 x 1.34 n 0.01 x 3.01 n 0.02	x 53.29 n 1.52
Hyla annectens	x 0.17 n 0.01	x 0.32 n 0.07	x many n -	x 0.37 n 0.02	x 4.80 n 0.10	x 1.81 n 0.00 x 2.78 n 0.09	x 57.48 n 0.52
Polypedates maculatus	x 0.39 n 0.05	x 2.43 n 0.39	x 105.00 n 22.22	x 0.36 n 0.02	x 4.06 n 0.13	x 0.73 n 0.00	x 58.29 n 1.71
Euphlyctis cyanophlyctis	x 1.12 n 0.37	x 3.57 n 0.49	x 9.00 n 4.44	x 0.33 n 0.03	x 4.36 n 0.23	x 0.78 n 0.00 x 1.42 n 0.00	x 62.03 n 1.71
Rana alticola	x 0.02 n 0.01	x 0.08 n 0.01	x 14.00 n 3.37	x 0.30 n 0.10	x 1.57 n 0.05	x 0.73 n 0.01	x 45.47 n 0.61
Polypedates leucomystax	x 0.18 n 0.03	x 10.37 n 1.55	x 13.00 n 2.23	x 1.44 n 0.02	x 3.82 n 0.13	x 2.50 n 0.18	x 50.46 n 4.78
Amolops formosus	x 1.84 n 0.52	x 8.80 n 0.42	x 21.00 n 9.00	x 1.42 n 0.05	x 2.71 n 0.06	x 2.07 n 0.00 x 2.45 n 0.00	x 52.70 n 1.62
Bufo melanostictus	x 0.10 n 0.01	x 0.13 n 0.01	x 105.00 n 19.18	x 0.34 n 0.04	x 4.01 n 0.13	x 1.69 n 0.01	x 59.21 n 1.34
Hoplobatrachus tigerinus	x 0.29 n 0.08	x 1.01 n 0.29	x 16.00 n 1.00	x 0.30 n 0.02	x 4.32 n 0.14	x 0.52 n 0.02 x 1.65 n 0.03	x 61.09 n 0.65

x = mean; n = standard deviation

white or creamish. Throat grey, sometimes black granulate.

3. Morphological measurements

Absolute measurements of 15 morphological characters were recorded from 10 males and 10 females respectively.

4. Call characteristics

The calls are given in rapid succession. Each call lasts about 0.110 s with 56 pulses, given at intervals of approximately 0.252 s. The call has a single dominant frequency at about 1.08 kHz extending from 0.35 kHz to 4.40 kHz. The sound pressure level (SPL) of the call being 68.1 dB.

Limnonectes khasiana (Figs. 2ABC and 11; Tables 1 and 2)

1. Habit and habitat

It is found in rice fields and grasslands.

2. Colouration

This is a tiny reddish brown species. Dorsal side with brownish triangular dark patches, ventral surface light brown with dark patches.

3. Morphological measurements

Absolute measurements of 15 morphological characters were recored from 4 males (3 are adult and 1 young froglet). The data from females have not been taken into account, since the number of females collected were less than 5.

4. Call characteristics

The calls are loud and given in rapid succession. Each call lasts about 0.151s with

Figure 2.

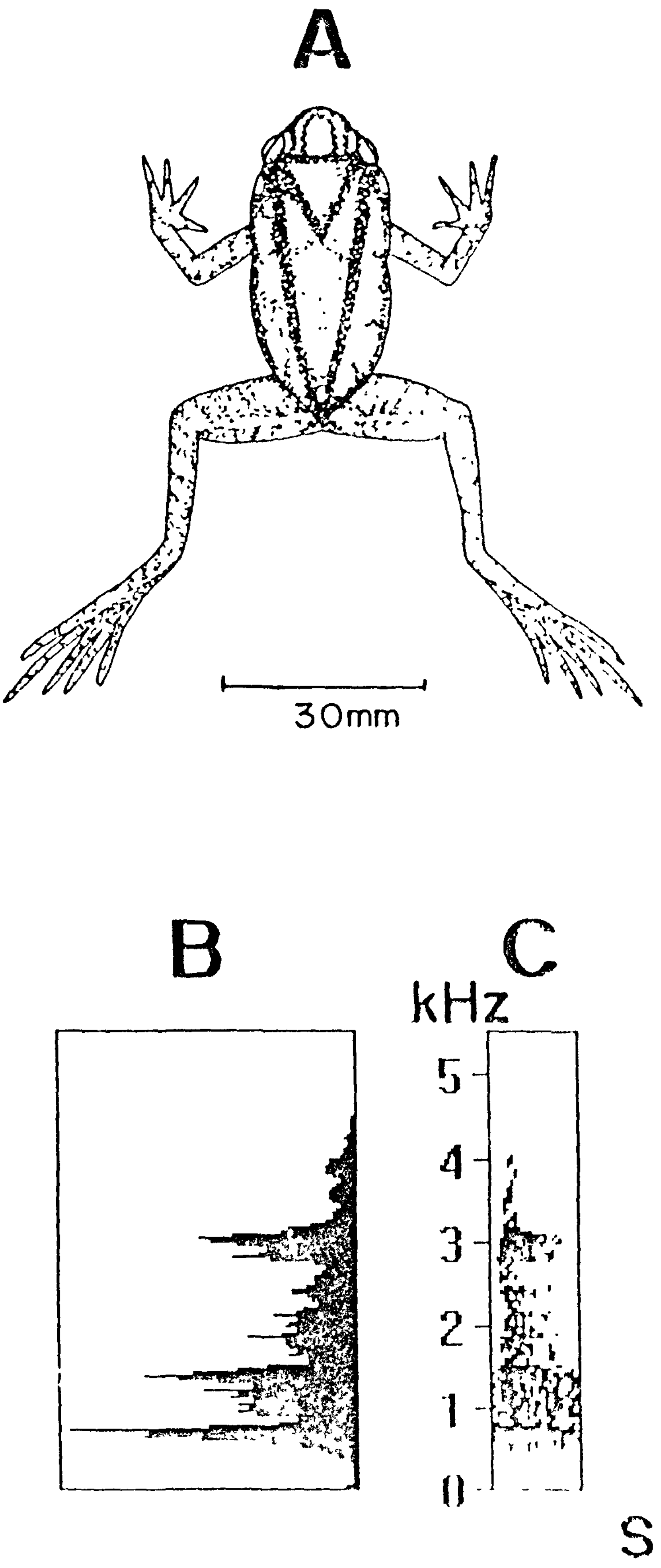
A. Line diagram of *Limnonectes khasiana* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

2



LIMNONECTES KHASIANA

many pulses given at approximately 0.219s. It is a harmonic call having dominant frequencies at about 0.67 kHz, 1.34 kHz and 3.01 kHz.The frequency domain lies in the range of 0.35kHz to 4.20 kHz. The SPL of the call being 53.29 dB.

Hyla annectens (Figs. 3ABC and 11; Tables 1 and 2)

1. Habit and habitat

Commonly known as the garden frog, mainly found in potato fields and in the garden climbing from one tree to another. Out of the 260 valid species of this genus from the world, it is only species found in northeast India.

2. Colouration

Dorsal surface dark green with a light brown streak from eyes to nostrils. A black lateral streak present upto groin often terminating into two black spots of different sizes with interconnections. Ventral surface of the thigh yellowish. A few black spots arranged more or less in line present on the ventral surface of the femur and tibia.

3. Morphological measurements

Absolute measurements of 15 morphological characters were recored from 7 males and 5 females.

4. Call characteristics

The calls are loud and noisy. Each call lasts for about 0.166s with many pulses, given at intervals of 0.321s. The call is harmonic having dominant frequencies at about 1.81 kHz and 2.78 kHz . The frequency domain extends from about 0.37 kHz to 4.80 kHz. The SPL of the call being 57.48 dB.

Figure 3.

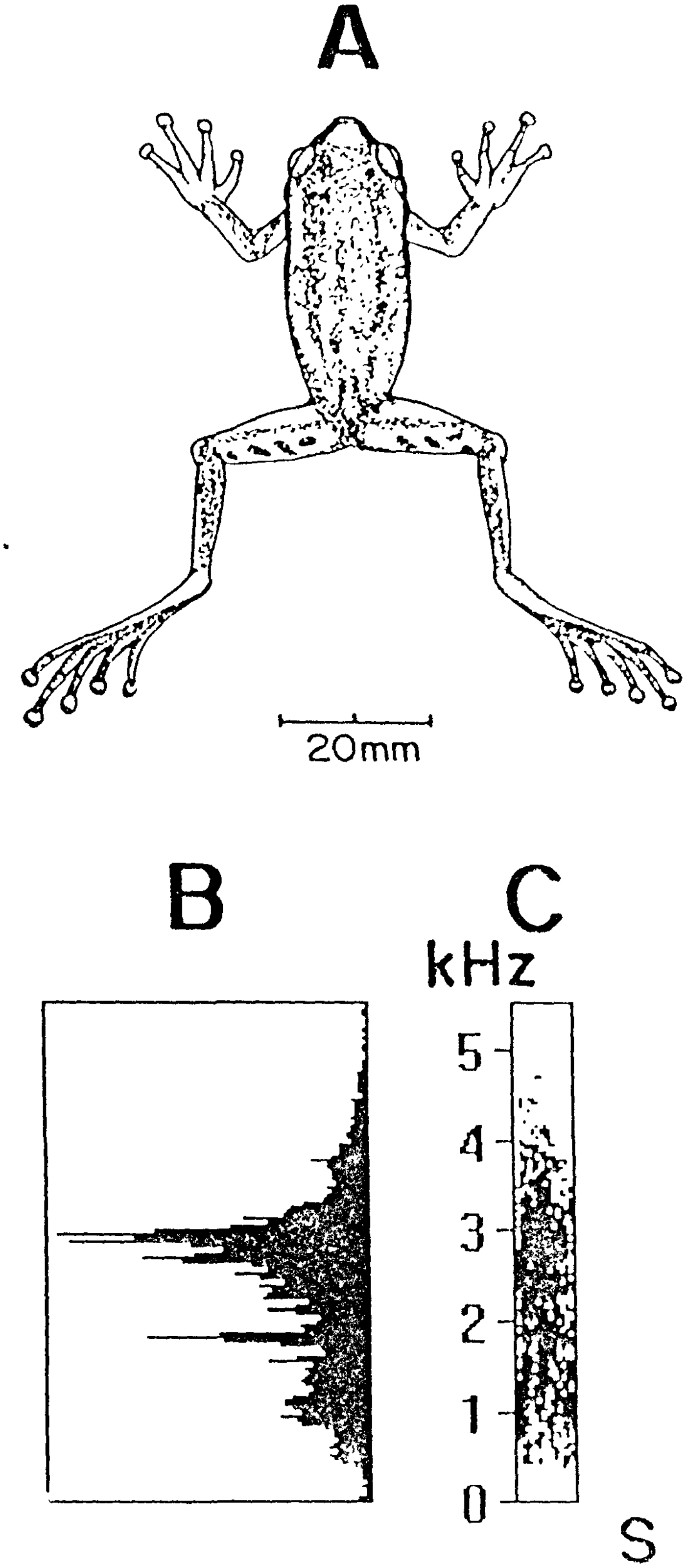
A. Line diagram of *Hyla annectens* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

3



HYLA ANNECTENS

Polypedetes maculatus (Figs. 4ABC and 11; Tables 1 and 2)

1. Habit and habitat

The frog is found in paddy fields and marshy grassland. It is also found among potato and bean plantation. The calling males conceal themselves under potato leaves or other vegetation.

2. Colouration

Dorsally yellowish brown to dark brown. Limbs with brown and white cross bars of irregular patterns. Thigh and throat are light brown to yellowish ; the chin and throat are with dark large oval spots.

3. Morphological measurements

Absolute measurements of 15 morphological characters were recorded from 10 males and 6 females.

4. Call characteristics

The calls are loud and distinct. At the initiation of the call, the calls have two distinct components, with the passage of time the components go on increasing in number. Each call lasts for about 0.389s, having almost as many as 105 pulses, given at intervals of 2.427s. The call has a single dominant frequency at about 0.73 kHz, the frequency domain extending from about 0.36 kHz to 4.06 kHz. The SPL of the call being 58.29 dB.

Euphlyctis cyanophlyctis (Figs. 5ABC and 11; Tables 1 and 2)

1. Habit and habitat

Frogs are aquatic in nature. Found in pools, muddy swamps and canals. These frogs can remain in water for long without coming on to the land and spend most of their time

Figure 4.

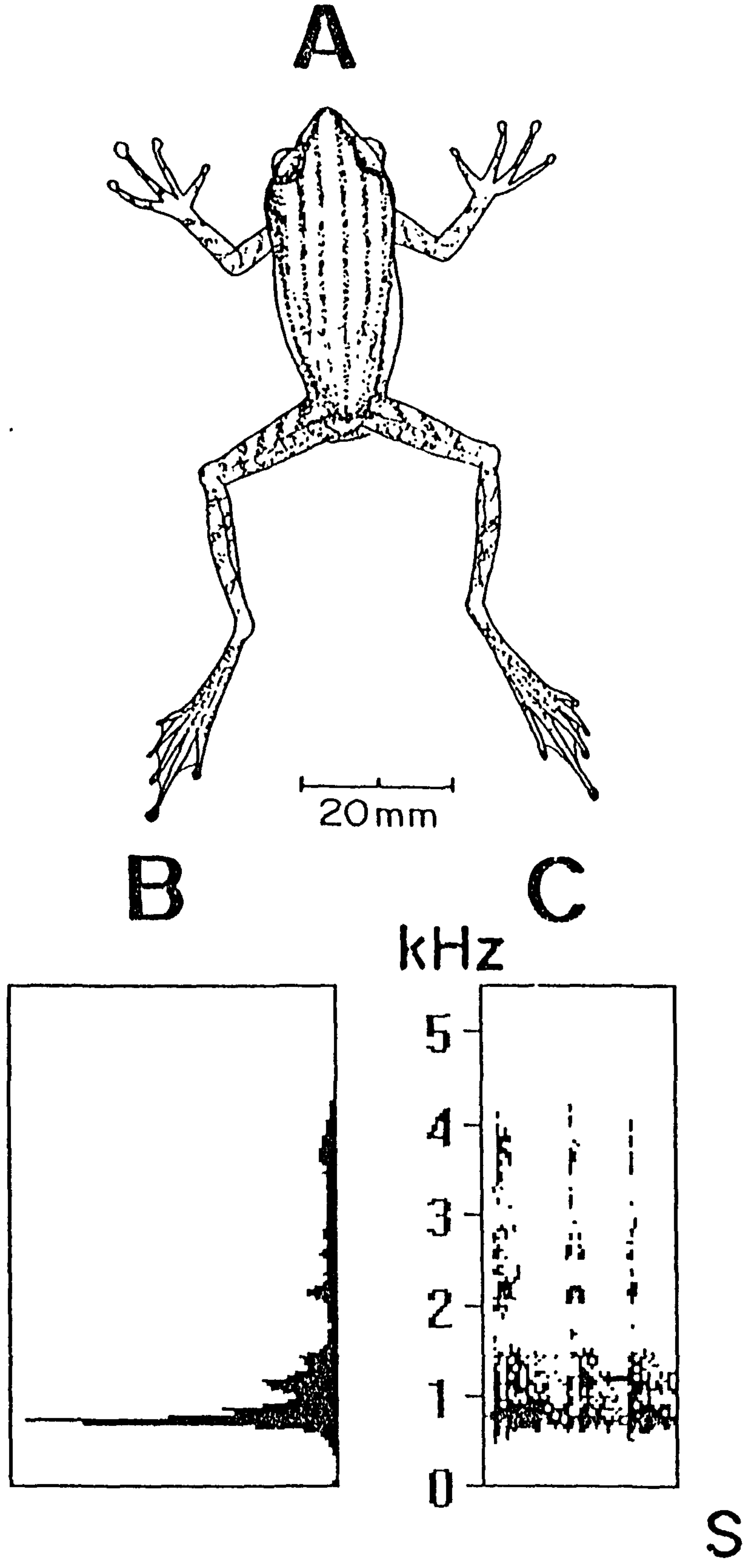
A. Line diagram of *Polypedates maculatus* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

4



POLYPEDATES MACULATUS

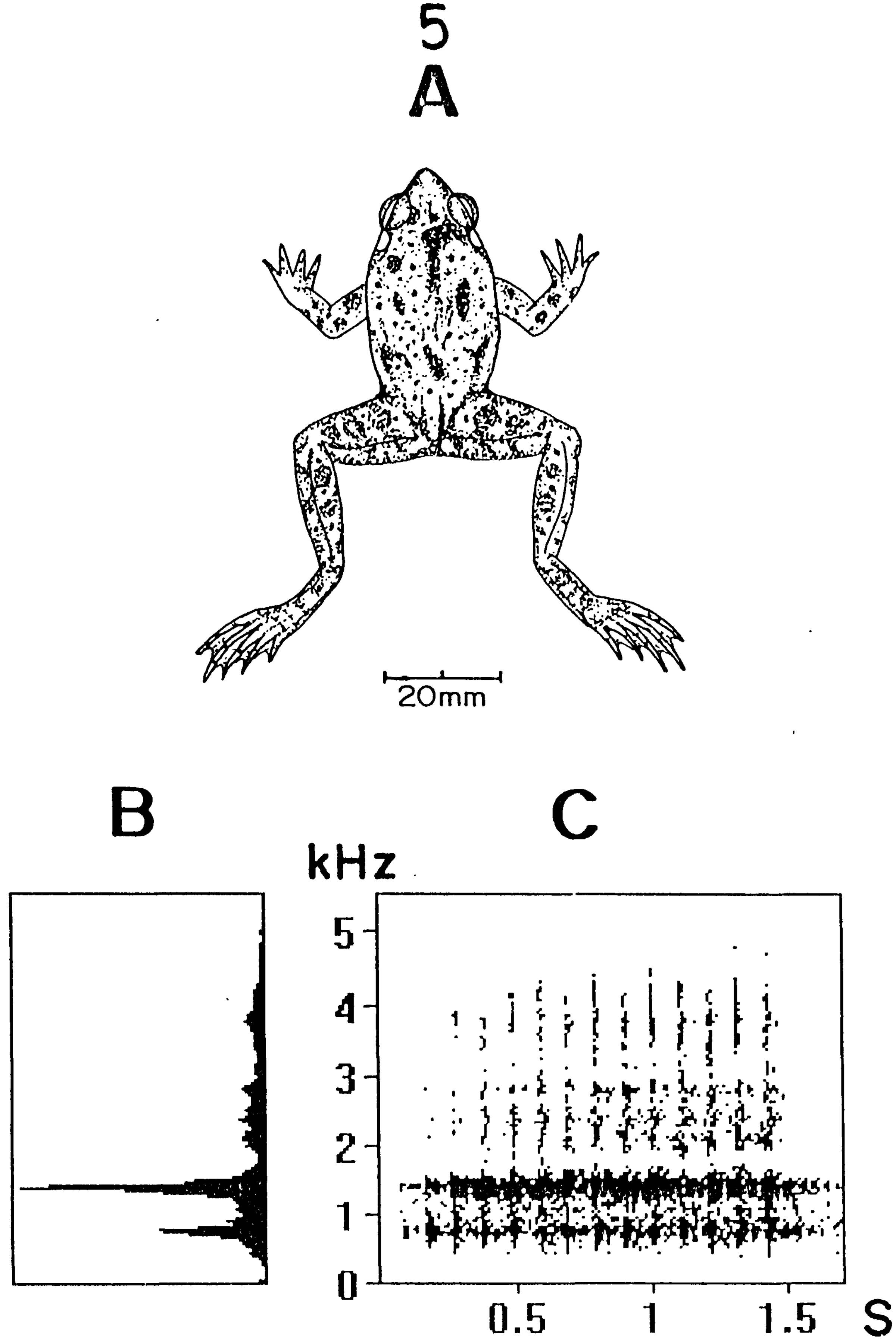
Figure 5.

A. Line diagram of *Euphlyctis cyanophlyctis* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)



EUPHLYCTIS CYANOPHLYCTIS

floating motionless, with eyes and tip of the snout above water. When alarmed they skitter across the water surface for several feet before diving and hiding on to the bottom.

2. Colouration

Dorsal surface light olive green or brown to almost black with irregularly arranged sooty spots, posterior surface of thigh dark often with one or two yellow or white, irregular, longitudinal stripes. Ventral surface is white. Vocal sacs are dusky.

3. Morphological measurements

Absolute measurements of 15 morphological characters have been completed for 10 males and 10 females.

4. Call characteristics

The calls have distinct pulse components, which goes on increasing with the passage of time. Each call lasts for about 1. 122 s, having about 9 pulses given at a call interval of about 3.574 s. The call is harmonic having dominant frequencies at about 0.78 kHz and 1.42 kHz. The frequency domain extends from about 0.33 kHz to 4.36 kHz. The SPL being 62.03 dB.

Rana alticola (Figs. 6ABC and 11; Tables 1 and 2)

1. Habit and habitat

Found in ponds, ditches, beels and low lying areas abundant in aquatic vegetation.

2.Colouration

Dorsally yellowish to light brown. Two distinct glandular dorsolateral folds running from the anterior region of the body to the posterior ending near the groin.

Figure 6.

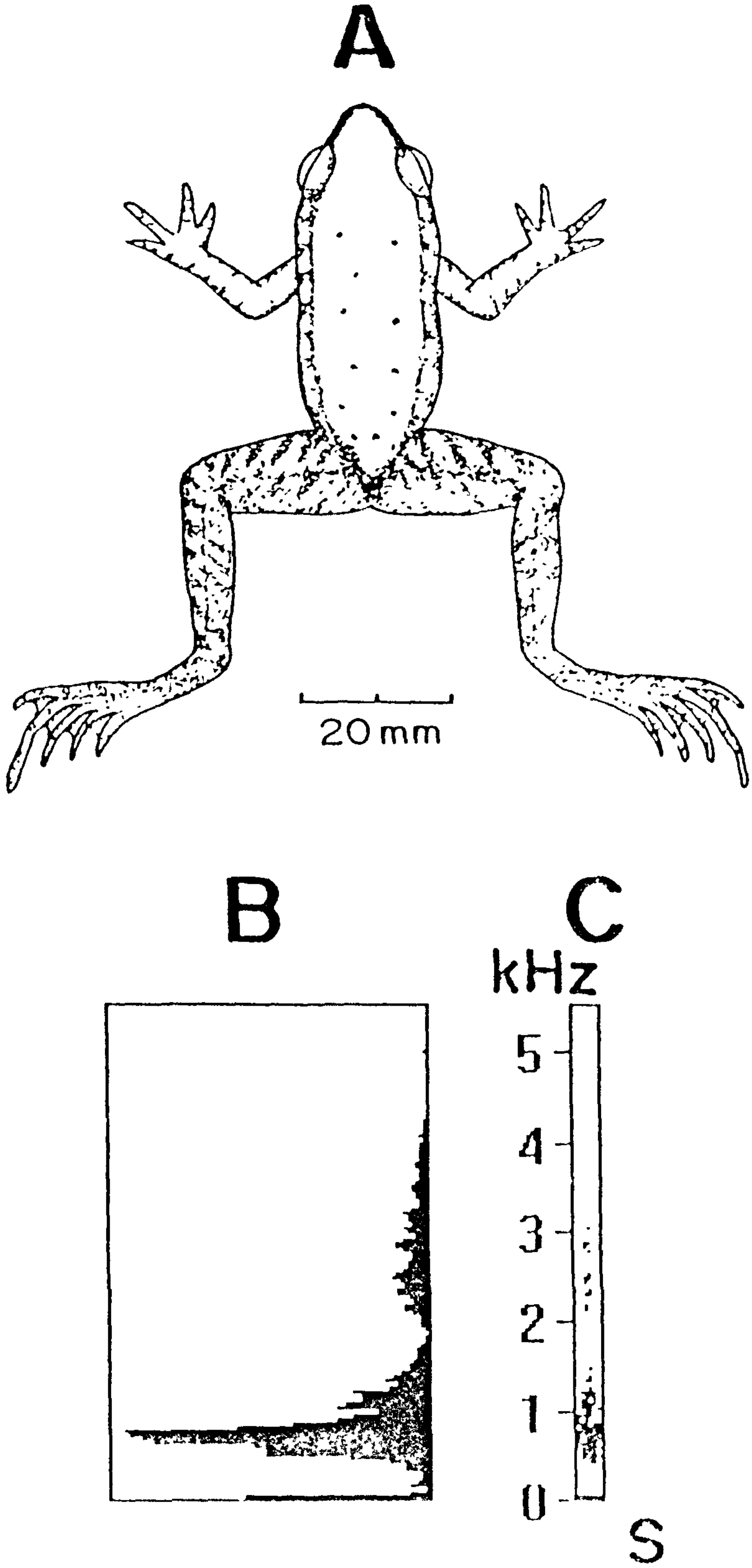
A. Line diagram of *Rana alticola* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

6



RANA ALTICOLA

3.Morphological measurements

Absolute measurements of 15 morphological characters have been compiled for 7 males and 6 females.

4. Call characteristics

The calls are repeated very rapidly. Each call lasts for about 0.020 s having approximately 14 pulses given at a call interval of 0.079 s. The call has a single dominant frequency at about 0.73 kHz, the frequency domain lies between 0.30 kHz to 1.57 kHz. The SPL of the call being 45.47 dB.

Polypedates leucomystax (Figs. 7ABC and 11 ; Tables 1 and 2)

1. Habit and habitat

Mostly collected from Assam. They are found on creepers , entwining bamboo fencing or tall grass near the vicinity of water.

2. Colouration

Dorsal surface and sides olive to yellowish green, broad light coloured stripes on dorsolateral folds running between eyelids and groin. Ventral surface is cream coloured. Dorsal surface of legs olive brown with dark marking arranged longitudinally in lines.

3. Morphological measurements

Absolute measurements of 15 morphological characters have been compiled for 10 males and 10 females.

4. Call characteristics

Distinct well spaced calls. The total call duration being approximately 0.182 s with about 13 pulses given at an interval of about 10.369 s. The call has a single

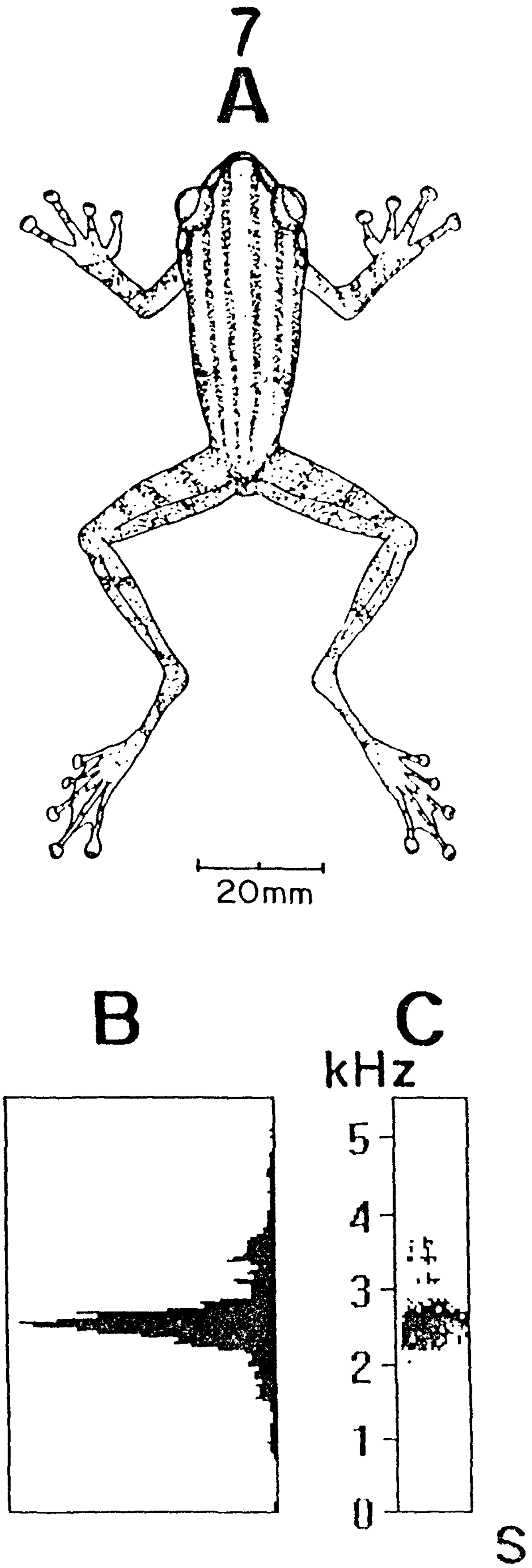
Figure 7.

A. Line diagram of *Polypedates leucomystax* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)



POLYPEDATES LEUCOMYSTAX

dominant frequency at about 2.50 kHz and the frequency domain extends from 1.44 kHz to 3.82 kHz. The SPL of the call being 50.46 dB.

Amolops formosus (Figs. 8ABC and 11; Tables 1 and 2)

1. Habit and habitat

Frogs were found both by the sides of streams as well as undwelled forest caves.

2. Colouration

Green with black patches on dorsal surface. Ventrally creamish with black patches mostly concentrated in lower jaw and throat regions.

3. Morphological measurements

Absolute measurements of 15 morphological characters have been compiled for 10 males and 10 females.

4. Call characteristics

These calls can be mistaken as insect calls. The call is a long continuous trill. Sometimes the call consists of two components, a main trill ending with a beep. The call duration is about 1.840 s, having about 21 pulses. With the passage of time number of pulses increase. The call is given at an interval of 8.802 s. It is a harmonic call with dominant frequency at about 2.07 kHz and 2.45 kHz. The frequency domain extends from 0.30 kHz to 4.32 kHz. The SPL of the call being 52.7 dB.

Bufo melanostictus (Figs. 9ABC and 11; Tables 1 and 2)

1. Habit and habitat

This species is found throughout the year. They are found mainly on land under stones and damp places.

Figure 8.

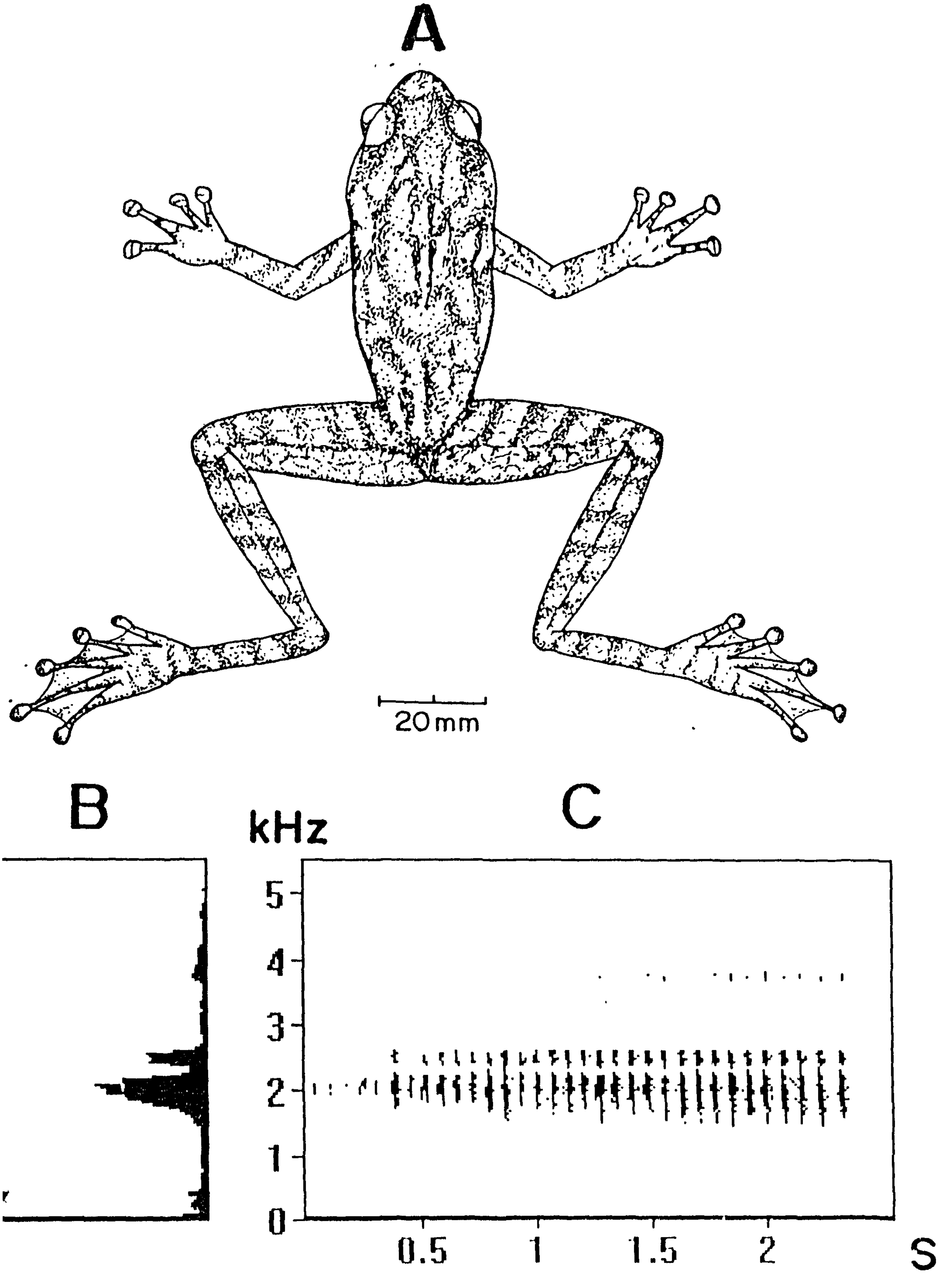
A. Line diagram of *Amolops formosus* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

8



AMOLOPS FORMOSUS

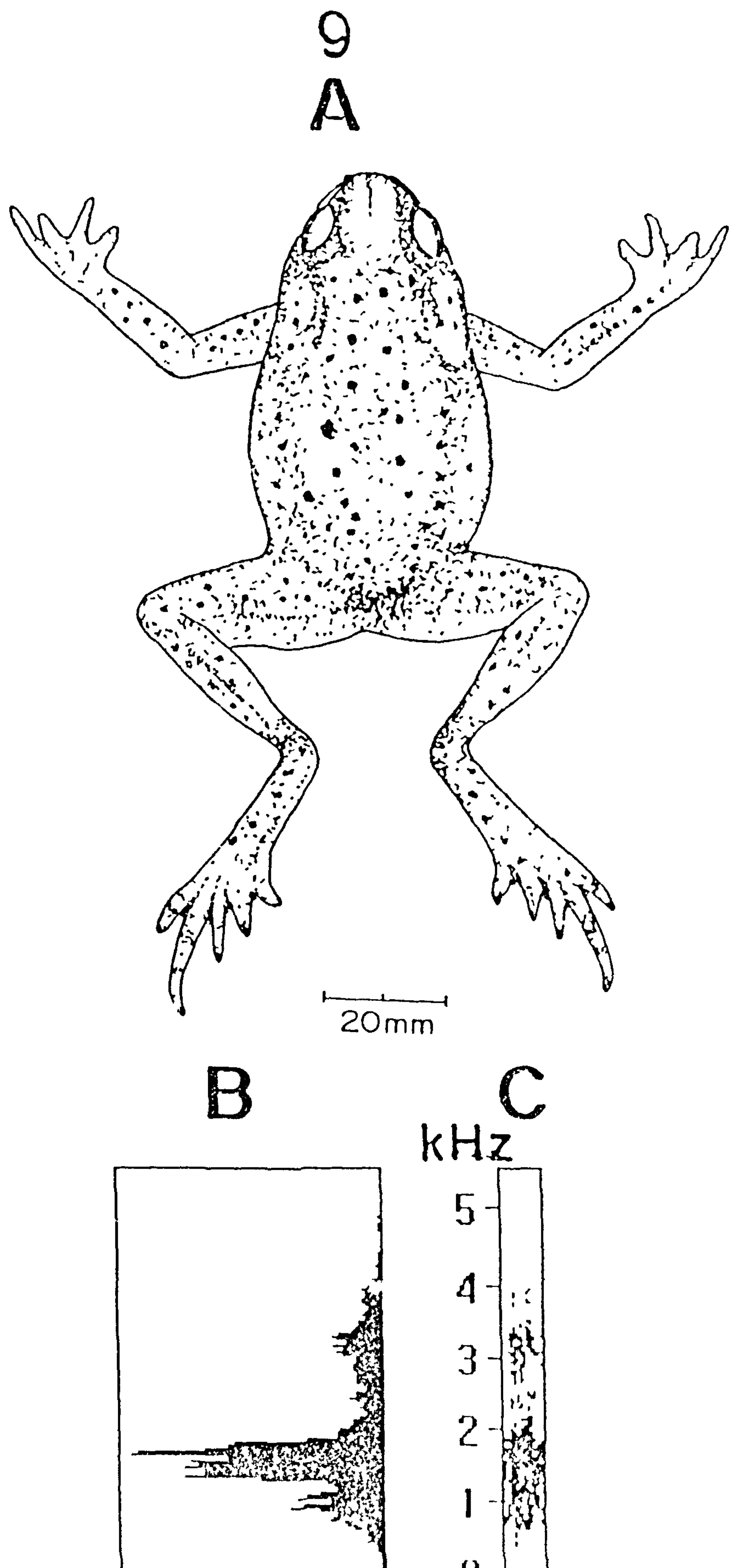
Figure 9.

A. Line diagram of *Bufo melanostictus* (to scale).

B. Mean spectra of the mating call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)



BUFO MELANOSTICTUS

2.Colouration

Brown to yellowish brown, dorsally with black spots. Tips of warts and ridges of head usually deep brown black. Ventrally creamish at times with light brown spots.

3. Morphological measurements

Absolute measurements of 15 morphological characters have been compiled for 10 males. Data from females have not been taken into account, since the number of female collected were less than 5.

4. Call characteristics

The loud croaks can be heard from a distance. These are rapidly repeated calls. The call duration being approximately 0.103 s with as many as 105 pulses, given at intervals of about 0.134 s. The call has a single dominant frequency at about 1.69 kHz. The frequency domain extends from about 0.34 kHz to 4.01 kHz. The SPL of the call being 59.21 dB.

Hoplobatrachus tigerinus (Figs. 10ABC and 11; Tables 1 and 2)

1. Habit and habitat

Commonly known as tiger frog. They are always found near water with weed choked ponds, ditches, tanks and marshes. During monsoon it is widespread in flooded low lands. They are mostly found singly. On sunny days it often spends hours crouched in grass or at the entrance of drains and culverts.Although these frogs are strong jumpers and swimmers, it is very easy to catch them since they keep sitting motionless for hours together.

2. Colouration

The dorsal colouration of the adult is light brown to olive, with grey or brown spots. Younger frogs paler in colour mostly grass colour. Distinct, narrow, cream coloured stripe

Figure 10.

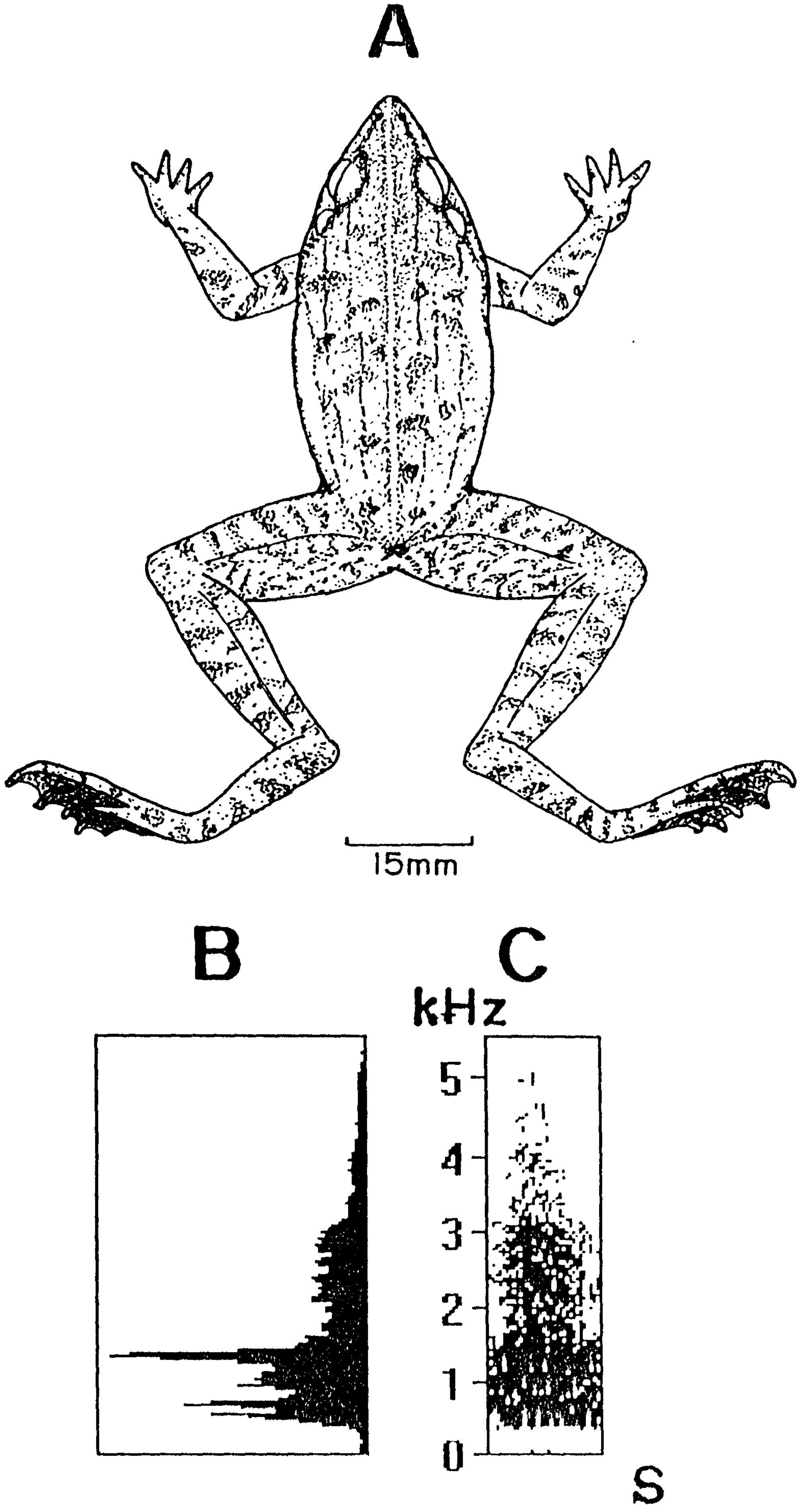
A. Line diagram of *Hoplobatrachus tigerinus* (to scale).

B. Mean spectra of the advertisement call.

C. Sonagram of the mating call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

10



HOPLOBATRACHUS TIGERINUS

from snout to vent and light line along upper surface of thigh and posterior aspect of tibia to heel.

3. Morphological measurements

Absolute measurements of 15 morphological characters have been compiled for 10 males. Data from female frogs have not been taken into account since the number of females collected were less than 5.

4. Call characteristics

These frogs have deep hoarse calls. The call duration being about 0.290 s with 16 pulses and call interval being approximately 1.011 s. It is a harmonic call, with dominant frequency at about 0.52 kHz and 1.65 kHz. The frequency domain extending from 0.30 kHz to 4.32 kHz. The SPL of the call being 61.09 dB.

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Chapter - 4

INTRODUCTION

Having studied the individual call pattern of adults we studied the intra and interspecific signal interaction using playback experiments or phonotaxis.

Playback is an experimental technique commonly used to investigate the significance of signals in animal communication systems. It involves replaying recordings of naturally occuring or synthesised signals to animals and noting their response. Playback has made a major contribution to our understanding of animal communication. Communication occurs when a signal from individual, the sender, alters the behaviour of another individual, receiver. The signal resulted from some structure or act specially adapted for the purpose, rather than as a by product of some other behaviour. The effect of the signal benefits the sender and may benefit the receiver.

(a)

```
                                   CONTEXT
                                      +
SENDER-------------------------------- SIGNAL --------------------- RECEIVER
signal      message                   +         meaning            response
          ( broadcast               NOISE                        ( transmitted
            information               +                            information
            encoded )               REDUNDANT                      decoded )
```

(b)

```
                                   CONTEXT

                                      +
                                   PLAYBACK-------------- SUBJECT
                                      +         meaning       response
                                    NOISE      ( inferred )  ( observed )
                                      +
                                   OBSERVER
```

Fig. 1 : Communication process - (a) Natural , (b) Experimental.

Playback is essentially an analytical tool for investigating the sufficiency of sound. A related consideration is that playback assists us in studying only certain aspects of the communication process. We can manipulate the structure of the signal and observe the response but we are dependent on observation, including signal analysis, for the rest.

Nearly all of the acoustic research on amphibians has been done with frogs and toads. There are many paralleles with insect studies, including the concentration on female responses to mating calls of mates.

Playback to amphibians began in the late 1940's. In 1947 Bogert reported playing a chorus of southern toads, *Bufo terrestris* attracted both males and females. A decade later, Martof and Thompson (1958) showed the females were attracted to both natural and synthetic calls of male chorus frogs, *Pseudacris nigrita* . The following year, Littlejohn and Michand (1959), using a two speaker design, demonstrated the female chorus frogs , *Pseudacris streckeri* and *P. clarki* could discriminate between calls of their own species and those of another sympatric species. Similar result was obtained with two closely related allopatric species , *Pseudacris streckeri* and *P. ornata* by Blair and Littlejohn (1960).

In subsequent studies, the response of females was used to analyse the effective components of male song (or synthetic copies), e. g. of bull frogs , *Rana catesbiana* by Capranica (1966) and in green tree frogs *Eleutherodactylus coqui* of Puerto Rico whose "coqui" call has different frequency components addressed to males (the " co " part) and females (the " qui "). Responses of each sex were obtained by Narins and Caprnica (1976) in a series of playback experiments using partial and complete calls. The pulse rate of frog calls vary with temperature. Using two sympatric treefrogs, *Hyla versicolor* and *H. chrysoscelis*, whose calls differed in this parameter, Gerhardt (1978) showed that the responses of females were temperature dependent so that they tracked the changing pulse rate of the male calls of their own species.

Intraspecific variation in anuran calls has given rise to a number of studies using playback to investigate the possibilities of sexual selection through female choice and male-male encounters. Within a species, larger individuals tend to have deeper calls and several investigations have been based on this phenomenon. Ryan (1981) showed that the females of the Tungara frog , *Physalaemus postulosus* prefer to approach calls of low, rather than high, fundamental frequency. The larger males that give deeper calls enjoy greater mating success than do smaller males. Searcy and Anderson (1986) review other studies in which female responses varied with calling rate or calling order of males. Gerhardt (1982) investigated sound pattern recognition in treefrogs (Hylidae) and has recently (1991) reviewed the female mate choice. Bullfrogs have territorial calls that elicit aggression in other males (Wiewandt 1969). A case involving differential male responses was reported by Davis and Halliday (1978). Presented with playbacks of calls from large and small conspecifics, common toads, *Bufo bufo* showed fewer and shorter attacks in response to the deeper " croaks " of the large individuals. Two other topics have been investigated in treefrogs, *Hyla* sp. using playback - (a) energetics of calling (Wells and Taigen 1986) and (b) graded signalling (Wells and Schwartz 1984). Thus, a wide range of acoustic behaviour has been investigated in amphibian using playback.

In this part of the study, it was aimed to find out the intra and interspecific interactions between species using playback experiments. The experiments were designed to answer the following questions :

1. Is the response direction dependent ?

2. Is the response distance dependent ?

3. Species response to conspecific and heterospecific signals.

4. To find the species relatedness.

To find out the answers of the above questions, playback experiments were done taking 6 test signals, 6 test species, 4 directions and 3 distances.

MATERIALS AND METHODS

Six different species *Limnonectes limnocharis, Euphlyctis cyanophlyctis, Polypedates maculatus, Polypedates leucomystax, Rana alticola* and *Bufo melanostictus* were collected during the breeding season (1994 - 1996). Their calls (advertisement) were recorded on a professional SONY WM - 6 DC cassette recorder with an unidirectional AKG C451 EB shotgun condenser microphone held approximately 40 - 60 cm away from the calling frog. The calls were stored in Maxell XLII cassette tapes. Sound pressure level was measured by playing back isolated calls on a Philips double cassette player DR 920 with play back volume fixed at 3 and the CYGNET 2021 sound pressure level meter held approximately 1m away from the sound source. The experiments were conducted at a constant temperature of 22^0 C (± 2). Three morphological characters weight (gm), snout-vent-length (cm) and tympanum diameter (mm) were recorded for both test and signalling species. When the signalling species did not call spontaneously, they were induced to call by playing back their recorded calls. On hearing the recorded call most of the frogs start advertising, specially during breeding season (Plates 1A and B).

The playback arena of 6 x 3 ft. was made of sound proof pressed board. It was covered with black polythene sheet. To visually measure the direction and distance moved by the test species, the black polythene sheet was manually marked by flouroscent markers into small squares of 1 sq. inch (Plate 2). The speaker were placed as per requirement of the experiment. A circle of 4 inch diameter was marked in the centre of the arena, where the test species was to be placed. The test species was initially covered with a glass funnel, while the test signal was played back for 2 mins. This was done to acquaint the test species to the test signals and at the same time restrict their immediate random movement. After 2 mins the funnel was removed. Any movement by the test species within 5 mins of funnel removal is considered as a response. The responses have been classified as :

a. **Positive response (+)** when the test species move towards the sound source.

b. **Negative response (-)** when the test species moved away or in the opposite direction of the sound source.

c. **No response (NR)** for non-displacement of the test species.

A score of 1 was assigned to all positive responses, - 1 to all negative responses and 0 to " no response " cases.

The experimental distances were taken as 10 cm, 20 cm and 30 cm. These distances were choosen with the logic that females are found to produce their feeble reciprocal call,

Plate 1 A.

Male *Limnonectes limnocharis* attracted towards the cassette player and listening to the playback call.

Plate 1 B.

More *Limnonectes limnocharis* males attracted towards the cassette players and listening to the playback call ; the male which was attracted first response by producing calls (← inflated vocal sacs during calling).

PLATE -1

A


SONY
CUE MARKER
SLIDING
MICROPHONE
V.O.R
V·O·R

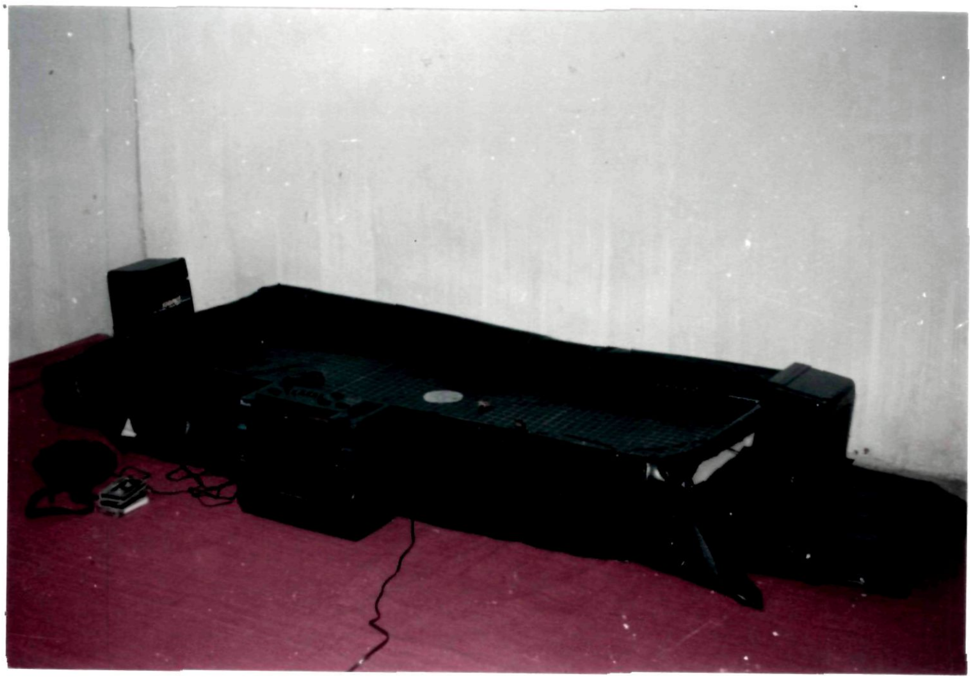
B


SONY
CUE MARKER
SLIDING
MICROPHONE
V.O.R
V·O·R
FAST PLAYBACK
AUTO SHUT OFF
CUE & REVIEW
TAPE COUNTER

Plate 2.

Arena for conducting playback experiments.

PLATE-2



when the distance between the signalling males and the responding female is 10 cm. The males are found to change their call pattern when the intermale distance reduces to 30 cm.

Unlike the usual ANOVA where the response variable was continuous , here , the response variable was discrete. The responses were either +ve, -ve or NR (no response) type. Therefore response variable and 3 classes - one class for each respose type.

As data belonged to 3 classes, we used the accumulation analysis technique for the 3-way ANOVA. This technique consists of first determinig the frequency of occurence for each class. For experiments with equal number of trials for each factor, the cumulative frequency would be the same in the 3rd class i. e. 3rd class would show no variation. Hence in acumulation analysis, the number of classes is analysed as -1. It is required to calculate a weight for the remaining two classes. This weight is a function of the cumulative frequency of occurence of that class. The formulae for the weight for class ***i*** is

$$W\,i = \frac{N_2}{T\,i\,(N - T\,i)} \qquad i = 1, 2$$

Where **T *i*** = accumulative occurence for class ***i***

N = total number of trials in the experiment.

Three different sum of squares (direction, distance and species) are determined. The sum of square for each factor is the weighted sum of squares for each class for the factor.

In our experiment, calls of all the 6 species were played one after the other from all the 4 directions (A, B, C and D) and at three different distances (x = 10 cm, y = 20 cm and z = 30 cm). Six different experiments were performed. In each such experiment, signal of a particular species was played and responses of 6 different species were recorded. The data were then tabulated for six 3-way ANOVA - one for each signalling species.

OBSERVATIONS

The observed behavioural displays by the 6 test species to 6 different test signals, from 4 directions and 3 distances were recorded and tabulated for statistical analysis. All the test values were calculated at 5 % probability level.

In Table 1A, degree of freedom, sum of square and F-test values of 3 different sources, i.e. species, direction and distance have been tabulated. Degree of freedom for the 3 sources were 5, 3 and 2 ; sum of squares were 3.14, 0.13 and 2322.14 and F-test values were 0.945, 0.064 and 1763.00 respectively. The table also has included the error in degree of freedom and sum of squares, 1717 and 1130.59 respectively.

Table 1B, the sum of squares of 3 different factors (direction, distance and species) in relation to 6 different signalling species *Rana alticola, Polypedates maculatus, Euphlyctis cyanophlyctis, Polypedates leucomystax, Limnonectes limnocharis and Bufo malanostictus* have been tabulated. The degree of freedom were 3, 2 and 5 respectively. The sum of squares in relation to 3 different factors for the different species were as followes : 2.43, 472.05 and 1108.02 respectively. In *P. maculatus* 4.35, 761.01 and 917.24; for *E. cyanophlyctis* the sum of squares were 0.81, 872.09 and 883.35 and in *P. leucomystax* 0.91, 385.95 and 846.17; *L. limnocharis* 2.94, 777.88 and 993.17 and *B. malanostictus* 1.55, 368.29 and 904.17. No significant interaction was found for direction for all 6 species. Interactions were significant in response to distance.

Table 1A. 3-way ANOVA test for receiving and calling species.

Source	Degree of freedom	Sum of squares	F - test
Species	5	3.14	0.95
Direction	3	0.13	0.06
Distance	2	2,322.14	1763*
Error	1,717	1,130.59	
Total	1,727	3,456	

* Significant at 5% probability level.

Table 1B. Sum of squares.

Factor	Differences in response due to direction	Differences in response due to distance	Response species (Response by 6 different species)
Degree of freedom	3	2	5
When the signalling species was -			
Rana alticola			
Sum of squares	2.4337	472.0544	1108.0209
5% probability level	No	Yes	Yes
Polypedates maculatus			
Sum of squares	4.346	761.01	917.24
5% probability level	No	Yes	Yes
Euphlyctis cyanophlyctis			
Sum of squares	0.811	872.09	883.35
5% probability level	No	Yes	Yes
Polypedates leucomystax			
Sum of squares	0.909	385.95	846.17
5% probability level	No	Yes	Yes
Limnonectis limnocharis			
Sum of squares	2.9403	777.88	993.17
5% probability level	No	Yes	Yes
Bufo melanostictus			
Sum of squares	1.552	368.29	904.17
5% probability level	No	Yes	Yes

Table 2A, the response scores by 6 different species at different distances (x = 10 cm, y = 20 cm and z = 30 cm) have been tabulated. At 10 cm all the 6 species showed no response. At 20 cm the responses were as follows : *L. limnocharis* 52, *E. cyanophlyctis* 49, *P. maculatus* 10, *R. alticola* -13, *B. melanostictus* -32 and *P. leucomystax* -128. At 30 cm distance the 6 species showed negative values - 53 , -113 , -102 , -62 , -75 and -126 for *L. limnocharis, E. cyanophlyctis, P. maculatus, R. alticola, B. melanostictus* and *P. leucomystax* respectively.

Table 2B, the test signal of *R. alticola* was tested for responses by the 6 test species. At 10 cm for all species there was no response. At 20 cm conspecific showed the highest value 38, followed by *E. cyanophlyctis* 16, *B. melanostictus* -12, for both *P. maculatus* and *P. leucomystax* the value was -17 . *L. limnocharis* -21. At 30 cm for conspecific the value was -10 and for heterospecific -18, -6, -9, -9 and -10 for *E. cyanophlyctis, B. melanostictus, P. maculatus, P. leucomystax, L. limnocharis* respectively.

Table 2C showed the test signal of *E. cyanophlyctis* which responded by 6 test species. At 10 cm all the species have showed no response. At 20 cm conspecific showed highest value 50, followed by *P. maculatus* 44 , *B. melanostictus* 32, *P. leucomystax* -20, *L. limnocharis* -21 and *R. alticola* -36 respectively . At 30 cm for conspecific the value was -4 and for heterospecific -12, -22, -8, -8 and -59 for *P. maculatus, B. melanostictus, P. leucomystax, L. limnocharis* and *R. alticola* respectively.

Table 2D showed the test signal of *P. maculatus* was tested for 6 test species . At 10 cm there was no response for all the species. At 20 cm conspecific species has showed

Table 2A. Response scores at different distances.

Signalling species	Distance x (10cm)	y (20cm)	z (30cm)
Euphlyctis cyanophlyctis	0	49	-113
Polypedates maculatus	0	10	-102
Bufo melanostictus	0	-32	-75
Polypedates leucomystax	0	-128	-126
Limnonectes limnocharis	0	52	-53
Rana alticola	0	-13	-62

Table 2B. Response scores at different distances when the signal was of *Rana alticola.*

Species placed at the centre of the arena	x (10cm)	Distance y (20cm)	z (30cm)	Total response score for each species
Rana alticola	0	38	-10	28
Limnonectes limnocharis	0	-21	-10	-31
Euphlyctis cyanophlyctis	0	16	-18	-2
Bufo melanostictus	0	-12	-6	-18
Polypedates maculatus	0	-17	-9	-26
Polypedates leucomystax	0	-17	-9	-26
Total response score at different distances	0	-13	-62	-75

Table 2C. Response scores at different distances when the signal was of *Euphlyctis cyanophlyctis.*

Species at the centre of the arena	Distance x (10cm)	y (20cm)	z (30cm)	Total response score for each species
Euphlyctis cyanophlyctis	0	50	-4	46
Polypedates maculatus	0	44	-12	32
Polypedates leucomystax	0	-20	-8	-28
Limnonectes limnocharis	0	-21	-8	-29
Rana alticola	0	-36	-59	-95
Bufo melanostictus	0	32	-22	10
Total response score for each distance	0	49	-113	-64

Table 2D. Response scores at different distances when the signal was of *Polypedates maculatus.*

Species placed at the centre of the arena	Distance x (10cm)	y (20cm)	z (30cm)	Total response score for each species
Polypedates maculatus	0	36	-16	20
Limnonectes limnocharis	0	24	-30	-6
Polypedates leucomystax	0	-24	-9	-33
Rana alticola	0	-19	-9	-28
Euphlyctis cyanophlyctis	0	16	-28	-12
Bufo melanostictus	0	-23	-10	-33
Total response score for each distance	0	10	-102	-92

highest value 36 , followed by *L. limnocharis* 24, *E. cyanophlyctis* 16, *R. alticola* -19, *B. melanostictus* -23 and *P. leucomystax* -24 respectively. At 30 cm for conspecific was -16 and for heterospecific -30, -28, -9, -10 and -9 for *L. limnocharis, E. cyanophlyctis, R. alticola, B. melanostictus* and *P.leucomystax* respectively.

Table 2E showed the signal of *B . melanostictus* for 6 test species. At 10 cm there was no response for all the species. At 20 cm. conspecific showed 30 , followed by *L. limnocharis* 6, *E. cyanophlyctis* 0, *P. leucomystax* -22, *P. maculatus* -23 and *R. alticola* -23 respectively. At 30 cm for conspecific the value was -6 and for heterospecific -20, -20, -11, -7 and -11 for *L. limnocharis, E. cyanophlyctis, P. leucomystax, P. maculatus* and *R. alticola* respectively.

Table 2F showed the signal of *P. leucomystax* for 6 responding species. At 10 cm. for all species there was no response. At 20 cm. conspecific showed the highest value 30, followed by *B. melanostictus* -23, *R. alticola* -25, *P. maculatus* -35, *L. limnocharis* -37 and *E. cyanophlyctis* -38 respectively. At 30 cm for conspecific the value was -18 and for heterospecific -10, -14, -28, -28 and -28 for *B. melanostictus, R. alticola* , *P. maculatus, L. limnocharis* and *E. cyanophlyctis* respectively.

Table 2G showed the signal of *L. limnocharis* for 6 test species. At 10 cm for all species there was no response. At 20 cm *B. melanostictus* showed the highest value 44 , followed by *P. maculatus* 40, *L. limnocharis* 30, *R. alticola* -15, *P. leucomystax* -23 and *E. cyanophlyctis* -24 respectively. At 30 cm for *B. melanostictus* the value was -2 and for heterospecific -14 , -10 , -8 , -9 and -10 for *P. maculatus* , *L. limnocharis, R. alticola* , *P. leucomystax* and *E. cyanophlyctis* respectively.

Table 2E. Response scores at different distances when the signal was of *Bufo melanostictus*.

Species placed at the centre of the arena	x (10cm)	Distance y (20cm)	z (30cm)	Total response score for each species
Bufo melanostictus	0	30	-6	24
Euphlyctis cyanophlyctis	0	0	-20	-20
Limnonectes limnocharis	0	6	-20	-14
Polypedates maculatus	0	-23	-11	-34
Polypedates leucomystax	0	-22	-11	-33
Rana alticola	0	-23	-7	-30
Total response score for each distance	0	-32	-75	-107

Table 2F. Response scores at different distances when the signal was of *Polypedates leucomystax*.

Species placed at the centre of the arena	Distance x (10cm)	y (20cm)	z (30cm)	Total response score for each species
Polypedates leucomystax	0	30	-18	12
Polypedates maculatus	0	-35	-28	-63
Limnonectes limnocharis	0	-37	-28	-65
Rana alticola	0	-25	-14	-39
Euphlyctis cyanophlyctis	0	-38	-28	-66
Bufo melanostictus	0	-23	-10	-33
Total response score for each distances	0	-128	-126	-254

Table 2G. Response scores at different distances when signal was of *Limnonectes limnocharis*.

Species placed at the centre of the arena	Distance			Total response score for each species
	x (10cm)	y (20cm)	z (30cm)	
Limnonectes limnocharis	0	30	-10	20
Polypedates maculatus	0	40	-14	26
Polypedates leucomystax	0	-23	-9	-32
Rana alticola	0	-15	-8	-23
Euphlyctis cyanophlyctis	0	-24	-10	-34
Bufo melanostictus	0	44	-2	42
Total response score for each distance	0	52	-53	-85

Table 3 showed the overall comparison of all the tables. When the signalling and responding species were *R. alticola*, it showed highest value 28. When *R. alticola* signal was tested for heterospecific interaction i. e. *E. cyanophlyctis, P. maculatus, P. leucomystax, L. limnocharis* and *B. melanostictus* the value were -95, -28, -39, -23 and -30. In case of *E. cyanophlyctis* conspecific scored 46 and for heterospecifics i. e. *R. alticola, P. maculatus, P. leucomystax, L. limnocharis* and *B. melanostictus* values were -31, -12, -66, -34 and -20. For *P. maculatus* conspecifics value was 20. Among heterospecific highest value was of E. *cyanophlyctis* 32 followed by *L. limnocharis, R. alticola, B. melanostictus, P. leucomystax* having values 26, -26, -34 and -63 respectively. In case of *P. leucomystax*, conspecific value was 12. *R. alticola, E. cyanophlyctis, P. maculatus, L. limnocharis and B. melanostictus* showed -26, -28, -33, -32 and -33 respectively. When the signalling and receiving species was *L. limnocharis* value was 20 . For *P. maculatus* , *B. melanostictus* , *E. cyanophlyctis* , *R. alticola, P. leucomystax* values were -6, -14, -29, -31 and -65. In case of *B. melanostictus, L. limnocharis* obtained highest value 42. *B. melanostictus* obtained 2nd highest value 24 and *E. cyanophlyctis* got 3rd highest value 10. *P. maculatus* and *P. leucomystax* both obtained -33 and *R. alticola* obtained -18.

DISCUSSION

Playback can be used either in the laboratory or in the field. There is more precise control of the conditions and measurement in the laboratory but precision does not necessarily imply accuracy. Specially for large and mobile animals, the laboratory is not a natural context. The field , with all its unknown and uncontrolable variables - is where

Table 3. Response scores for different species (Scores computed after observing response to 288 calls for each calling and receiving species).

Responding or receiving species	Signalling species					
	Rana alticola	*Euphlyctis cyanophlyctis*	*Polypedates maculatus*	*Polypedates leucomystax*	*Limnonectes limnocharis*	*Bufo melano - stictus*
Rana alticola	28	-95	-28	-39	-23	-30
Euphlyctis cyanophlyctis	-31	46	-12	-66	-34	-20
Polypedates maculatus	-26	32	20	-63	26	-34
Polypedates leucomystax	-26	-28	-33	12	-32	-33
Limnonectes limnocharis	-31	-29	-6	-65	20	-14
Bufo melanostictus	-18	10	-33	-33	42	24

behaviour may be most uninhibited , natural and complete. Ideally we can have the best of both venues by linking laboratory and field studies. Playback helps us to do that. That is the reason, the playback experiment was used to study the inter and intraspecific interactions.

The results of the playback experiment showed clearly that the direction did not play any role in intra and interspecific interactions. On the contrary, distance played a - significant role in the response behaviour for all the 6 species tested. Distance and response by different species was significant at 5 % probability level (Tables 1A and B).

Again between the three distances tested, at position y (i. e. 20 cm) maximum interaction was observed when the interaction was tabulated between conspecifics which always +ve, whereas for heterospecifics, the responses were mixed , having both +ve and -ve values. From this +ve and -ve values species relatedness can be predicted (Table 2F).

Interesting phenomena was observed while recording inter and intraspecific interaction at distance y. For both *Polypedates maculatus* and *Bufo melanostictus* besides showing +ve scores for conspecific interactions, they showed relatedness with *Euphlyctis cyanophlyctis* and *Limnonectis limnocharis* by scoring positive values (Table 3). Although relatedness is shown by the 2 species *Euphlyctis cyanophlyctis* and *Limnonectis limnocharis,* but the ranking and closeness can only be predicted with the help of DNA profiles.

The response among the distances experimented upon, 30 cm distance always has -ve scores. More than 30 cm distance 10 cm distance is of great significance when all the responses scored " 0 " .

The female reciprocal call is given by the sexually mature, eager to mate, responding females to the male advertisement calls, only when the distance between the two interacting male and female reduces to 10 cm (Chapters 6 and 7). But when male-male interaction was studied during playback experiment at 10 cm " No response" or " 0 " was scored for each and every experiments. Thus the difference between male-female and male-male interaction at 10 cm distance raises the following questions :

1. Are the interactions different for males and females ?

2. Could the difference in interaction for the females be due to the larger body size and the resulting distance of the tympanum from the ground which makes the females receptive to the calls even at 10 cm interaction distance ?

Thus the playback experiment leads to significant results, some of which could be explained, whereas others need to be research upon in-depth.

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Chapter - 5

INTRODUCTION

Amphibia is an interesting class of vertebrates possessing the crucial and important event-transition from the aquatic to terrestrial form of life. Frogs and toads undergo extensive change during their transition i. e. metamorphosis from tadpole to froglet stage. Inspite of the evolutionary, adaptive and physiological significance of the transition period, it is very surprising that the amphibians in general remain the least studied class in the vertebrates. Amphibian biologists still remain as the minority community among the biologists worldwide (Philips 1994). It is more so for the behavioural biologists.

Inspite of India's rich amphibian biodiversity there are very few workers on amphibians in the country (Chanda 1986; 1988; 1990a, b, c; 1991; 1994; Dutta 1985-1987; 1990a, b, c; Kiyasetuo and Khare 1986; Kripalani 1961; Mansukhani and Sarkar 1980 - 1981; Mohanty-Hejmadi 1974; Murthy 1967-1968a, b; Pillai 1976-1978; 1980; 1981a, b; 1986; Kanamadi 1992; 1993a, b; 1994; 1996; Roonwal and Kripalani 1951; Roy and Elepfandt 1993; Roy 1994; Roy et al. 1995; Roy et al. 1998). Thus realising the importance of amphibians of northeast and at the same time the meager amount of work done on the amphibians of northeast, the present study was planned.

Survey of literature reveals that although there are number of studies in the recent past on amphibian auditory system and acoustic communication in amphibians in the west as well as in India, but all studies remain confined to the adult call pattern and their related behavioural displays (Capranica 1976; Gerhardt 1982; Hetherington and Lombard 1982; Littlejohn 1977; Roy and Elepfandt 1993; Roy 1994; Roy et al. 1995; Roy et al. 1998; Ryan 1986; Wells 1977; Wever 1973). There are few works on premetamorphic or early post metamorphic stages but are mostly confined to morphological feature of the ear of the transforming larva (Paterson 1949; Roy and Elepfandt 1990; Sedra and Michael 1959; Spaeti 1978; Weisz 1975; Witschi 1949) or kin recognition in tadpoles (Blaustein and O'Hara 1982; Blaustein, Bekoff and Daniels 1987; Blaustein and O'Hara 1988; Waldman and Adler 1979; Waldman 1981). But no work has been done on the Indian species.

Amphibians undergo extensive change during metamorphosis, when the aquatic larval period preceedes the terrestrial period. Their locomotor and respiratory organs become entirely remodeled. They develop new nutritional habits and accordingly the digestive system suffers radical change. One of the most spectacular changes which takes place during metamorphosis are the morphological and functional change from underwater hearing in premetamorphic aquatic tadpoles to aerial hearing in adult terrestrial frogs accompanied by replacement of the larval accessory apparatus by entirely new tympanic organs of the adult. Thus, this change from aquatic to terrestrial habitat represent an astounding example of adaptation from water borne sound stimuli to air borne sound stimuli.

Thus transition from aquatic to terrestrial life was one of the most important events in the evolution of higher vertebrates. In the ontogeny of amphibians, the change from fish to tetrapod is faithfully repeated generation after generation. When vertebrates first ventured onto land, they had to adapt to terrestrial condition in various ways. It holds true also for hearing , because the properties of sound are quite different in air than in water. Fishes possess inner ear with otolith organs. Sound waves in water run through the body of the fish and vibrate its body. Air borne sound of normal intensity, however, will not bring the animal's head into vibration. As a result, the air filled middle ear develops for the first time in course of development.

The call patterns of adult frogs and the behavioural interactions have been studied. After studying the adult call pattern and their related behaviour it was felt necessary to find out the timing of development of the acoustically mediated behaviour.

In order to understand the development of acoustically mediated behaviour play back experiments were designed. The experimental subjects were the tadpoles leading through metamorphosis to young metamorphosed froglets till they attain sexual maturity. The significance of this particular time of development is, that, the tadpoles have a fish like hearing adapted to receive water borne acoustic stimuli. Tadpoles have inner ear like fish but no middle ear. Lungs develop at later stage and become connected to the inner ear through round window. This connection supports the hearing of tadpole similar to the way swimbladder support hearing in fish. The middle ear begins to develop during early metamorphosis. The connection between the lung and inner ear ends at metamorphosis. As metamorphosis sets in, the earlier organisation undergoes disintegration. Tympanum starts to develop for the first time in the vertebrate series.

Though the middle ear has developed fully in the young froglet, the size of the head is considerably smaller than in the adult frog. Since the frog ear acts as a pressure gradient receiver which reacts according to the pressure difference between the tympanum and sound impinging on the tympanum's inner side via the eustachian tube, thus the size of the head should also have a considerable impact on the hearing ability of the frog.

Besides, it was felt that it would also be interesting to study and report whether there is any difference between tadpoles maintained in isolated and non-isolated condition. There are no such reports on amphibians. Therefore the experiments were designed for tadpole grown under isolated and non-isolated condition. For this purpose the experiments were done in both isolated and non-isolated conditions.

Although it was initially planned to study the morphological characters during the development of ear structures, but with experience on adult behavioural pattern it was felt that we could study the timing of development of acoustically mediated behaviour by non-invasive methods. Thus it was planned to rear the tadpoles through metamorphosis to young froglets under isolated and non-isolated conditions. During this period, body length, tail length, body weight, tympanum diameter and age were recorded at fortnightly interval for all the individuals.

MATERIALS AND METHODS

Breeding colonies were raised in the laboratory. Fertilised eggs were obtained from these breeding colonies. 50 % of the fertilised eggs were raised in acoustically isolated condition where no adult frogs were reared. As a result the batch of embryos were totally isolated from any kind of sound. The rest 50 % were raised along with the adult frogs, so that they could hear the conspecific and heterospecific adult calls.

Measurements of morphological characters like body length, tail length, body weight and tympanum diameter for each individual of each group were recorded at fortnightly intervals. The data for these morphological measurements were then subjected to statistical analysis.

Two types of aquarium set-up were used for rearing tadpoles

a. Aquarium for rearing tadpoles

Substrate : Peat moss layers covered with fine sand or gravel.

Plants : *Polygonum sp.* , *Amannia, Limnophila, Lycopodium, Dicranopteris, Hypericum* , *Euriocaulon* and *Sphenomeris*.

Decoration : Hiding places near strategic plant arrangements, such as rock grottos or tangles of ariel roots.

Water is changed regularly in the aquarium for normal development. During the first 2 days after fertilization, water is changed twice daily and then until the transition to active feeding once a day and later once in 2 days. The tadpoles do not require constant aeration, but water plants such as - *Polygonum sp.* , *Amannia, Limnophila, Lycopodium, Dicranopteris, Hypericum* , *Euriocaulon* and *Sphenomeris* are useful in aquarium for oxygenation. For the rearing of tadpoles and frogs in the aquarium some important criteria were followed. They were kept in sufficient volume of water, 100 tadpoles in 5000 ml of water. We followed this volume of water for normal development, because if their density is higher the development of different individuals proceeds asynchronously and with a general delay. In this volume of water the tadpoles can be kept until metamorphosis having no delay in the process.

b. Aquarium for metamorphosed froglet

During metamorphosis when the froglets tend to leave the water and come up on land, very little water is filled in the aquarium. Then aquariums are inclined, so that a portion of the aquarium remains dry. The bottom is covered with filter paper so that froglet does not slip too much coming out of the water. Small pebbles and branches of houseplants are placed on the water free part of the bottom. The aquariums are covered with nets so that the metamorphosed frogs cannot jump out. During this period water is changed daily.

OBSERVATIONS

A . Relation between tympanum diameter (TD) and body length (BL)

The relationship between tympanum diameter (TD) - the dependent variable(y) and body length (BL) - the independent variable (x), seperately for isolated and non-isolated conditions of rearing were developed as two models. Then the two models were compared to find out if there existed any difference for the two rearing conditions. The models were also used to predict the tympanum diameter (y) using body length (x).

i. Model for isolated condition

Study of the tympanum diameter and body length data for isolated condition revealed that development of tympanum occured only when the body length exceeded 2.2 cm. Since tympanum was not observed for body length $\leq$ 2.2 cm, data were recorded only when body length was greater than 2.2 cm (2.2 cm was excluded), along with their corresponding tympanum diameter.

We considered a straight line fit of the form -

$$y = \beta_0 + \beta_1 x$$

Where **y** is the tympanum diameter in mm and **x** the body length in cm.

The fitted model -

$$\mathbf{y = -2.3587 + 1.211091x\ (0.12086)}.$$

(The figure in paranthesis indicate the standard error of the corresponding regression coefficient).

The corresponding calculated value of t was 10.02 which is highly significant at 5% probability level.

(5% table value of t for 42-2 = 40 degrees of freedom is 2.02). Hence x does have an important influence on y in the above model.

The R^2 (R = multiple correction coefficient) for the above model was 0.72 indicating that 72 % of the total variation could be explained by the model. The calculated F-statistics for testing the significance of this multiple correlation co-efficient was 50 % which is highly significant at 5 % probability level. (The degree of freedom is 2.39. The 5 % table value of F at this degrees of freedom is 3.23). This significance indicated that the model (as above) was successful in estimating y (i.e. T.D.) using x (i.e. B/L).

The above model is a polynomial of degree 1. We tried fitting a polynomial of higher degree to the same data. Improvement in R^2 was marginal. This was confirmed by the fact that while adjusted R^2 for the straight line model (which was used) was 0.713, the adjusted R^2 for the polynomial of degree 2 model was lower, 0.706. Hence there was no justification in fitting a polynomial of higher degree.

ii. Model for non-isolated condition

Study of tympanum diameter and body length data for non-isolated condition revealed that development of tympanum occured only when the body length was 2 cm. Tympanum was found positive only when body length exceeded 2 cm.

A scatter diagram of this data revealed a non-linear relationship. Hence a quadratic model of the following form was considered :

$y = \beta_0 + \beta_1 x + \beta_2 x^2$

When y is the tympanum diameter in mm and x is the body length in cm.

The fitted model -

$$y = -10.104081 + 7.6848\,x - 1.31424\,x^2$$
$$(1.4809) \quad (0.29069)$$

(The figures in parenthesis indicate the standard errors of the corresponding regression coefficients.)

The corresponding calculated t - statistics were 5.19 (7.6848 / 1.4809) and - 4.52 (-1.31424 / 0.29069) and hence both were significant at 5% probability level with degrees of freedom = 66 (5% table value of t at 66 d.f. = 2). This significance indicated that both x and x^2 have significant and important influence on the variable y.

The R^2 of the above model was 0.7621 indicating that 76.21 % of the total variation was explained by this model. The calculated F - statistic for testing the significance of the multiple correlation coefficient was 69.4 which is highly significant at 5 % probability level. (The degrees of freedom is 3.65. The 5 % table value of F at this degrees of freedom = 2.75).

The above model is a polynomial of degree 2. It was tried to fit a polynomial of higher degree but the corresponding increase in R^2 was marginal. Therefore fitting polynomials of higher degree did not make much sense when compared to a polynomial of degree 2. The adjusted R^2 confirmed this hypothesis.

iii. Comparison between isolated and non-isolated model

The question which was addressed now was - Whether the 2 models were different ?

For this, dummy variables were used :

$\mathbf{y = a_1 + a_{12}\, D_2 + b_1\, x + b_{12}\, D_2\, x + C_1\, D_1\, x^2}$

Where $\mathbf{a_1}$ - the intercept.

$\mathbf{a_{12}}$ - regression coefficient of the dummy variable D_2 . The significance would indicate the intercept of the 2 models to be different. Its non-significant would indicate no difference.

$\mathbf{D_2}$ - a dummy variable which has -

value of 1 if data pertains to isolated cases

value of 0 if data pertains to non-isolated cases.

$\mathbf{b_1}$ - regression coefficient of x (denoting body length in cm).

$\mathbf{b_{12}}$ - regression coefficient of a variable which is the product of D_2 and x. Its significance would indicate significant difference between the coefficient of x in non - isolated case and the slope of x is isolated ones . Its non-significance would indicate no difference between the coefficient of x in the 2 models.

$\mathbf{C_1}$ - regression coefficient of a variable which is the product of D_1 and x^2 . The variable x denotes body length and D_1 the dummy variable having the value 1 for non-isolated cases and 0 for isolated cases.

For running regression analysis, data pertaining to the 2 models were combined. The independent variables were D_2 , x, $D_2\,x$ and $D_2\,x^2$. The dependent variable was y (tympanum diameter in mm). The fitted model was

$$\mathbf{y = -\ 10.10408 + 7.74538\ D_2 + 7.6848\ x - 6.4737\ D_2\ x - 1.31424\ D_1\ x^2}$$

$$\mathbf{(1.9367)\qquad (1.5112)\qquad (1.5157)\qquad (0.2966)}$$

The R^2 for the above model was 0.75.

The calculated t-statistic for the coefficients of D_2, x, D_2x and $D_1 x^2$ were 3.9, 5.08, - 4.27 and 4.43 respectively. They were significant at 5 % probablity level. (5 % table value of t at 106 d. f. was 1.98). Hence it was calculated that the two models were significantly different. In other words, the relationship between tympanum diameter and body length were different under the two rearing conditions.

B. Relation between tympanum diameter (T D) and body weight (W T)

After having developed a model between tympanum diameter and body length with the aim to predict tympanum diameter using body length, now a model was developed for tympanum diameter (in mm) using weight (in gm). Here also tympanum diameter (y) was the dependent variable and weight (x) the independent variable.

i. Model for isolated condition

Study of the tympanum diameter and weight data for isolated condition revealed that tympanum was non-existent till weight was 2.1 gm. Tympanum could only be observed once the weight was $\geq$ 2.2 gm.

The R^2 values for different types of models like polynomial, expontial etc. were not satisfactory. A careful study of the data revealed that weight decreased around the 22nd - 23rd fortnight and then increased. Whereas no decrease was observed in the corresponding tympanum diameter. Thus, the R^2 was low and not satisfactory.

Consequently, a new independent variable was introduced-age in fortnight (observations recorded on 1.4.95 was assigned the fortnightly age of 1, observations of

16.4.95 as 2 and so on .....) .

With the above modifications , the following model was fitted to the data :

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_1^2 + \beta_3 x_2 + \beta_4 x_2^2$$

where y denotes tympanum diameter in mm

x_1 denotes weight in gm

and x_2 denotes age in fortnight.

β_0 , β_1 , β_2 , β_3 and β_4 were regression coefficients to be estimated.

The fitted model -

$$\mathbf{y = - 5.54438 + 0.79036\, x_1 + 0.04261\, x_1^2 + 0.357087\, x_2 - 0.00739\, x_2^2}$$

$$\mathbf{(1.5469) \quad (0.3015) \quad (0.09939) \quad (0.00205)}$$

The R for the above model was 0.69 indicating that 69 % of the total variation could be explained. The calculated value of the F-statistic for testing significance of this multiple correlation coefficient was 15 which is highly significant at 5 % probability level (5 % table value of F at 5.35 d. f. is 2.48). Hence it was concluded that this model was useful in predicting tympanum diameter (y).

ii. Model for non-isolated condition

Study of tympanum diameter and weight for non-isolated condition revealed that tympanum was non-existent till weight was 2 gm. Tympanum could only be observed once the weight exceeded 2 gm.

Various models were fitted to the above data, e.g. polynomials of different degrees, exponential curves etc. However the fit was not satisfactory as the R^2 values were low - 0.40

to 0.45. The reasons were the same as for isolated condition.

Thus the new variable age in fortnight was introduced and the following model was fitted to the data :

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_1^2 + \beta_3 x_2 + \beta_4 x_2^2$$

Where **y** denotes tympanum diameter in mm

x_1 denotes weight in gm

and x_2 denotes age in fortnight.

β_0, β_1, β_2, β_3 and β_4 were regression coefficient to be estimated. The fitted model -

$$y = -6.38197 + 1.57076\, x_1 - 0.15798\, x_1^2 + 0.35733\, x_2 - 0.00712\, x_2^2$$
$$(1.938) \quad (0.3943) \quad (0.0489) \quad (0.00106)$$

(The figures in parenthesis indicate the standard errors).

The R^2 for the above model was 0.77 indicating that 77 % of the variation could be explained by the model. The calculated value of F-statistic for testing significance of this multiple correlation coefficient was 43 which is highly significant at 5 % probability level (5% table value of F at 5, 55 d. f. is 2.38). Hence it was concluded that this model was useful in predicting tympanum diameter (y).

The adjusted R^2 value for a model with higher power of x_1 and x_2 was less than the adjusted R^2 value of the model which was considered. Hence any model with higher power of x_1 or x_2 was not been considered.

iii. Comparison between isolated and non-isolated model

Two models have already been developed - for isolated and non-isolated conditions.

Now both the models were compared to check whether they differed.

Let the model for isolated condition be -

$$y = \alpha_0 + \alpha_1 x_1 + \alpha_2 x_1^2 + \alpha_3 x_2 + \alpha_4 x_2^2$$

and for non-isolated condition -

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_1^2 + \beta_3 x_2 + \beta_4 x_2^2$$

Using dummy variables, the two models can be combined as

$$y = \beta_0 + (\alpha_0 - \beta_0) D_2 + \beta_1 x_1 + (\alpha_1 - \beta_1) D_2 x_1 + \beta_2 x_1^2 + (\alpha_2 - \beta_2) D_2 x_1^2 + \beta_3 x_2$$
$$+ (\alpha_3 - \beta_3) D_2 x_2 + \beta_4 x_2^2 + (\alpha_4 - \beta_4) D_2 x_2^2$$

Here $D_2 = 1$, if data pertains to isolated case.

$D_2 = 0$, if data pertains to non-isolated case.

Testing the significance of the estimated regression coefficients of D_2, $D_2 x_1$, $D_2 x_1^2$, $D_2 x_2$ and $D_2 x_2^2$ would indicate if the models were different. The fitted model was

$$y = -6.3819 + \underset{(3.312)}{0.83759}\, D_2 + \underset{(1.9658)}{1.57076}\, x_1 - \underset{(2.48)}{0.7804}\, D_2 x_1 - \underset{(0.3999)}{0.15798}\, x_1^2 + \underset{(0.497)}{0.20059}\, D_2 x_1^2$$
$$+ \underset{(0.0496)}{0.35733}\, x_2 - \underset{(0.109)}{0.00024}\, D_2 x_2 - \underset{(0.00108)}{0.00712}\, x_2^2 - \underset{(0.00228)}{0.00027}\, D_2 x_2^2$$

The calculated R^2 for the above model was 0.74.

The calculated t - statistic for the estimated regression coefficients of D_2, $D_2 x_1$, $D_2 x_1^2$, $D_2 x_2$ and $D_2 x_2^2$ were 0.25, -0.31, 0.4036, -0.0022 and - 0.12 , all of which were not significant at 5 % probability level (5 % table value of t at 92 d. f. is 1.98). Hence it was concluded that there exists no significance difference between the two models. Thus there exists no relationship between tympanum diameter, weight and age for both the conditions.

C. Growth chart of tympanum under isolated and non-isolated conditions

It was now aimed to study the growth or increase in tympanum diameter in 2 different rearing conditions - isolated and non-isolated. Tympanum diameter of specimens (n = 10 for each condition) were recorded for over 30 fortnights. Two tympanum diameter growth charts were prepared . Each chart had 29 rows and 10 columns for each condition .

The (1, 10) th cell of the Table represented the growth in tympanum between fortnight number 1 and fortnight number 2 , for specimen number 10. In general, the (i, j) th cell of the table represented the tympanum growth during the i th and (i + 1) th fortnight for the j th specimen (i = 1, 2, ... 29 and j = 1, 2, ... 10). There were 4 null hypothesis to test :

H_0 1 : No significant difference existed in the mean tympanum growth between the isolated and non-isolated conditions.

H_0 2 : No significant difference existed in the mean tympanum growth between the 29 fortnights.

H_0 3 : Growing condition and fortnights did not effect each other.

H_0 4 : No significant difference existed in the mean tympanum growth among different specimens.

It was obvious that the factor ' condition ' was more important than the factor ' fortnight ' as the primary aim was to study if mean tympanum growth were different in the isolated and non-isolated conditions.

The split-plot design enable to test the above hypothesis when one factor was more important than the other. *Accordingly, the whole plot treatments were the fortnights and the* sub-plot treatments were isolated and non-isolated conditions. The specimens were replications.

The following were obtained :

Total number of observations in the isolated group = 29 x 10 = 290

Total number of observations in the non-isolated group = 29 x 10 = 290

Total number of observation in the experiment = 290 + 290 = 580

Total number of all observations in the isolated group = 6.7

Total number of observations in the non-isolated group = 7.5

Grand total of all observations = 7.5 + 6.7 = 1.42.

From the above AOV table, it was obvious that only ' fortnight ' was significant at 5% probability level. All the others were not significant. Thus it could concluded :

i.There was no significant difference between the tympanum growth in the different conditions i.e. isolated and non-isolated conditions.

ii. There was no significant difference between the fortnightly tympanum growths.

iii. Fortnights and conditions work independent of each other. They were not inter-related as far as their effect on tympanum growth was concerned.

iv. There was no significant difference between the tympanum growth of different specimens (This conclusion confirms that the choice of specimens was done in an unbiased manner) (Table 1) .

D. Study of the effect of conditions on the age at which tympanum was first observed

Tympanum did not exist in early tadpoles. It was observed after a span of time. It would be examined if the isolation or non-isolation affects the average age at which

Table 1. The AOV table.

Source	Degree of freedom	Sum of squares	Mean squares	Calculated F	Table value of F at 5% level
Specimen (replication)	9	0.070	0.007	0.887	1.88
Fortnight (whole plot treatments)	28	1.254	0.044	5.090	1.52
Error 1	252	2.217	0.008		
Situation (Sub plot treatments)	1	0.000	0.001	0.152	3.84
Interaction (whole plot x sub plot)	28	0.172	0.006	0.850	1.52
Error 2	261	1.896	0.007		
Total	579	5.612			

tympanum was observed for the first time.

The null hypothesis to be tested :

Here in H_0 : There did not exist any significant differnce between the mean age at which tympanum was first observed in isolated and non-isolated specimens.

The age (in fornights) at which tympanum was first observed among non-isolated specimens were 15, 27, 17, 29, 30, 27, 17, 29, 30.

Here mean (x_1) = 24.55556 ,

n_1 = Number of observations = 9

S_1^2 = 39.52778 (the sample mean square)

The age (in fortnights) at which tympanum was first observed among isolated specimens was 28, 30, 18, 30, 17, 28, 27, 30.

Here x_2 = mean = 26.0

n_2 = No of observations = 8

S_2^2 = Sample mean square = 25.25002

t- test was used for testing the significance of difference between two means which was given by

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{S_p^2 \left(\frac{1}{n_1} + \frac{1}{n_2} \right)}} \sim t_{n1+n2-2}$$

Where S_p^2 was the pooled mean square. However, this test was valid under the assumption that the variance of the populations from which the 2 samples had been drawn were equal i. e.

$H_0 : \delta_1 = \delta_2$ For testing this F-statistic was used which was given by

$$F = \frac{S_1^2}{S_2^2} \sim F_{n1-1,\, n2-1}$$

Here $$F = \frac{39.52778}{25.25002} = 1.565$$

5 % table value of F at (8, 7) d. f. = 3.73. Hence the population variance were equal in the 2 populations. Now t-test can be conducted .

$$t = \frac{24.55556 - 26.0}{\sqrt{34.54819 \left(\frac{1}{9} + \frac{1}{8} \right)}} = -0.51$$

Therefore $| t | = 0.51$

5 % table value of t at 15 d. f. (9 + 8 - 2) was 2.131. Hence the null hypothesis was accepted and concluded that there was no significant difference between the mean age at which isolated and non-isolated specimens exhibit tympanum growth for the first time.

E. Study of body length in isolated and non-isolated conditions - comparison there of

Body length (in cm) of specimens were recorded over 30 fortnights seperately for isolated and non- isolated conditions (n = 10). Similar to the study on growth of tympanum, data were collected to find out whether body length differed between the isolated and non-isolated conditions.

As in case of tympanum, here too, it a fortnightly tympanum growth table was prepared seperately for the isolated and non-isolated specimens. Each of these tables had 29 rows and 10 columns. The (i , j) th cell of any one of these tables represented the growth in body length between the i th and (i + 1) th fortnight of the j th specimens. First the non-isolated condition was studied. A 2- way ANOVA was carried out (with one observation per cell). The factors were specimen and fortnights (Table 2).

From the table it can be concluded that :

i. Specimen sum of squares was not significant at 5% probability level. Hence it was concluded that there was no significant difference between the non-isolated specimens for their body length.

ii. Fortnight sum of squares was significant at 5% probability level. Hence it was concluded that in non-isolated condition, there existed significant difference in fortnightly body length growth.

A similar analysis i .e. the 2-way ANOVA was carried out for the isolated specimens (Table 3).

Hence it can be concluded that :

i. There was no significant difference in body length growth among specimens.

ii. There was no significant difference in fortnightly body length growth.

Since there was no significant difference among specimens for both isolated and non-isolated groups, classification due to specimens was disregarded. This enabled to examine if there existed significant difference among isolated and non-isolated cases as far as body length growth was concerned.

By disregarding specimen classification, it was carried out a 2-way ANOVA was done with 10 observations per cell . The 2 factors were ' condition ' , (non-isolated versus isolated) and fortnight. The (i , j) cell relates to data pertaining to body length between

Table 2 . 2-way ANOVA for the specimen and age under non-isolated condition .

Source	Degree of freedom	Sum of squares	Mean square	5 % F value	
				Calculated	Tabulated
Specimen	9	0.018	0.002	0.427	1.88
Fortnight	28	0.913	0.032	6.694	1.00
Error	252	1.227	0.005		
Total	289	2.160			

Table 3. 2-way ANOVA for the specimen and age under isolated condition.

Source	Degree of Freedom	Sum of squares	Mean squares	5% F value	
				Calculated	Tabulated
Specimen	9	0.027	0.003	0.633	1.88
Fortnight	28	1.249	0.044	8.950	1.00
Error	252	1.257	0.004		
Total	289	2.534			

the i th and the (i + 1)th fortnight and situations number j (j = 1 if non-isolated, 0 if isolated). Here i = 1, 2,..... 29 and j = 0, 1. Each of these cells contained 10 observations relating to fortnightly body length growth data for the 10 specimens (Table 4) .

Thus it was concluded that :

i. There was no significant difference in body length growth between the isolated and non-isolated specimens.

ii. There was no significant difference between fortnightly body length indicating that there existed significant difference in body length growth between various fortnights.

iii. Fortnights and interactions were dependent. They were in fact related. This was the reason that the first analysis showed that the model between tympanum diameter and body length were different in isolated and non-isolated conditions.

DISCUSSION

Most workers deal with various questions posed under acoustic communication in amphibians taking the adult animals as the experimental model system. The metamorphosing tadpole pose many interesting problems, specially in context to hearing. The entire body of the tadpole undergoes restructuring and reorganisation. This is due to their change from their aquatic to terrestrial model of life. An important event during this period is the development of tympanum and middle ear. When air borne sound vibrations hit the tympanum or external ear drum, the tympanum is set into motion. The movement of the tympanum creates pressure difference. As a result the vibrations through the rarefication and condensation of the sound pressure wave is carried through the middle ear to the inner ear. Thus the development of

Table 4. 2-way ANOVA for comparing growth under isolated and non-isolated condition .

Source	Degree of freedom	Sum of squares	Mean squares	5 % F value	
				Calculated	Tabulated
Condition	1	0.000	0.000	0.050	3.84
Fortnight	28	1.859	0.066	13.690	1.46
Interaction between situation and fortnight	28	0.304	0.011	2.230	1.46
Error	522	2.532	0.005		
Total	579	4.695			

tympanum is crucial to transmit the sound vibrations to the inner ear.

Thus it was realised that if the relationship between the growing tympanum diameter and body length, inclusive of the tail length in tadpoles be worked out, then the stage from which acoustically mediated behaviour develops can be predicted. It was with this objective a detailed study was done, observations were systematically recorded and subjected to stastical analysis.

Besides, in other vertebrate groups specially in birds, the rearing conditions have an important bearing on the development of acoustically mediated behaviour (Levin 1996). But no such report exists for amphibians . Therefore along with the recording of the above mentioned observation, the tadpoles were grown under 2 different conditions - isolated and non-isolated. It was aimed to study whether the development of tympanum, indirectly relating to development of acoustically mediated behaviour is different under the 2 conditions. The relationship between tympanum diameter and body length were developed as 2 models with the objective of predicting tympanum diameter using body length.

Study of the tympanum diameter and body length data for isolated condition revealed that development of tympanum occured only when the body length exceeded 2.2 cm. Since tympanum was not observed for body length $\leq$ 2.2 cm, data were recorded only when body length $>$ 2.2 cm, along with their corrsponding tympanum diameter. For non-isolated condition, study of tympanum diameter and body length revealed that development of tympanum occurred only when the body length was 2 cm. Tympanum diameter data could only be recorded when body length exceeded 2 cm.

Study of tympanum diameter and weight data for isolated condition revealed that tympanum was non-existent till weight was 2.1 gm. Tympanum could only be observed once the weight $\geq$ 2.2 gm. Consequently, a new independent variable was introduced - age in fortnight. Study of tympanum diameter and weight for non-isolated condition revealed that tympanum was non-existent till weight was 2 gm. Tympanum could only be observed once the weight > 2 gm.

Thus from the growth chart of tympanum under isolated and non-isolated condition, it can be concluded that :

(1) There is no significant difference between tympanum diameter growth in isolated and non-isolated condition.

(2) For meaningful results data should be recorded fortnightly.

(3) Fortnight and condition worked independent of each other showing no relationship or effect on tympanum growth.

(4) There was no significant intraspecific growth difference for tympanum diameter.

By studying the effect of conditions on the age at which tympanum was first observed, it was found that tympanum did not exist in early stage of development for tadpoles. There was no significant difference between the mean age at which tympanum was first obseved in both the conditions.

From the study of body length for isolated and non-isolated condition it can be concluded that :

(1) There is no significant differences in body length growth under both the conditions.

(2) It was found that body length growth showed significant difference at fortnightly

intervals.

(3) The 2 parameters fortnight and interaction although indepenedent but are related. This can be explained by the first analysis when the models between tympanum diameter and body length were different in isolated and non-isolated condition.

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Chapter - 6

INTRODUCTION

Animals often produce vocal signals for communication (Duellman and Trueb 1986; Ewing 1989; Farabaugh 1982; Otte 1977). In amphibians vocal signals have proved invaluable in studying systematic relationships (Blair 1964; Littlejohn 1981; Littlejohn and Watson 1985) and the process of sexual selection (Gerhardt 1982; Ryan 1991). Males of many amphibian anuran species produce species-specific complex sounds composed of numerous, closely spaced, often harmonically related components with predominant amplitudes that fall into distinct frequency bands, the locations of which are characteristic for each species. Male calls help to advertise their position, to defend their territories and to attract conspecific females to the breeding site, thus acting as an important determinant of mate choice (Gerhardt 1988; Rand 1988; Ryan 1988). Females recognize the calls of conspecific males, locate them within a chorus of the calling conspecific and heterospecific males and find a mate. Playback experiments have verified the importance of both the gross and fine temporal characters, as well as the spectral domain and spectral pattern of the call in a noisy acoustic environment (Gerhardt 1988). Thus, the use of species-specific temporal and spectral character is one strategy for reducing heterospecific interference and maintenance of reproductive isolation (Drewry and Rand 1983; Duellman and Pyles 1983; Narins and Zelick 1988).

Acoustic communication plays a significant role in the reproductive behaviour and breeding biology of amphibians (Wells 1988). Anuran vocalization has been classified according to the behavioural context in which the calls are elicited (Bogert 1960 ; Littlejohn

1977; Wells 1977). Most of the literature pertaining to anuran acoustic communication deals specifically with species-specific temporal and spectral characters of the male calls (Gerhardt 1988; Drewry and Rand 1983; Roy and Elepfandt 1993; Roy 1994; Schneider et. al 1993). Although the female vocalization had been reported as early as 1929, these reports were restricted only to the alarm, release and aggressive calls in *Rana silvatica* (Noble and Ferris 1929), *Rana pipiens* (Noble and Aronson 1942), *Rana esculenta* (Wahl 1969), *Colostethus inguanalis* (Wells 1980) and *Eleutherodactylus coqui* (Stewart and Rand 1991).

Reciprocal call given in response to the male advertisement call was reported in the genus *Tomodactylus* based on behavioural observations, with, no call recordings (Dixon 1957). Heinzmann (1970) working on *Alytes sp.*, recorded two types of male calls - mating call and distress call. This work gives a brief description of the oscillogram for the reciprocal calls of the females, with no details of temporal and spectral characteristics. Female calling has also been reported in *Rana ridibunda* (Frazer 1983) and *Rana virgatipes* (Given 1987). It is noteworthy to mention here that, though sketchy reports on female reciprocal calls existed (Dixon 1957). Since 1957, in-depth study to look into the role of female reciprocal call in the breeding biology of amphibians has started only recently. A reason for this could be that the sensation of hearing for human is most acute in the 2000 - 5000 Hz frequency range and the female calls occur much below this. Indeed, it is difficult to distinguish and record a female call, which is usually masked by the loud advertisement calls of the conspecific and heterospecific males in the vicinity.

MATERIALS AND METHODS

While studying acoustic communication and related behavioural displays in the courting frogs of northeast India , we observed that females of *Polypedates leucomystax, Limnonectes limnocharis* and *Euphlyctis cyanophlyctis* emitted reciprocal calls in response to male advertisement calls. Due to their feebleness and short call duration, these reciprocal calls would not have been noticed had they not been given in rapid succession, producing at times chirp-like calls, which attracted our attention. We then recorded and analysed the female reciprocal calls.

The calls were recorded during the active breeding period (May to August 1993 and 1994). In 1993 only behavioural observations were made and in 1994 the calls along with behavioural observations were recorded and analysed. The calls were recorded with a unidirectional AKG C45 1EB shotgun condenser microphone held approximately 40 -60 cm away from the calling female. The calls were recorded with a professional SONY WM-D6C cassette recorder and stored on Maxell XLII cassette tapes. Sound pressure level was measured by playing back the isolated female calls on a Philips double cassette player DR920 with playback volume level fixed at 4 and the CYGNET 2021 sound pressure level meter held approximately 60 cm away from the sound source.

Recorded acoustic stimuli were digitized via a Microsoft analouge-to-digital interface board on to an IBM PC and stored on diskettes. Oscillograms, sonagrams and mean spectra were prepared with a computerized Fast Fourier Trasformation (FFT) system after passing through band pass filters and printed with a laser printer.

RESULTS

P. leucomystax are mostly arboreal in nature and hence their calls and reproductive behaviour were easier to observe and record in comparison to the calls of *L. limnocharis* and *E. cyanophlyctis*. The latter two species called mostly sitting on land on the edge of a swamp and while hovering on water surface, respectively. Besides, these two species were more easily disturbed, and swam deep into the water at the slightest disturbance. Calls of *P. leucomystax* were recorded at Guwahati, Assam (200m ASL; 91.5′ E ; 26.1′ N). *L. limnocharis* and *E. cyanophlyctis* calls were recorded from colonies being maintained under near natural condition in the froggery adjacent to the Institute at Shillong, Meghalaya (1515m ASL; 90.7′ E; 24.0′ N).

The females of *P. leucomystax* (Plate 1) are usually seen emerging and approaching the calling males after about 30 min of vocal advertisement by the male. When the distance between the calling male and the approaching female reduces to about 10cm, the male which had been calling till then stops advertising. The female makes feeble reciprocal calls in response to the male advertisement call. This feeble call of the female at times continues upto 1 hour, with several bouts of call. The interval between each bout varies from 2 to 5 min, short intervals at the beginning and long intervals towards the end of the call. Only after the female responds to the male advertisement call, the male which had stopped calling on the appearance of the female restarts calling with a higher intensity. Concomitantly, all the other neighbouring calling males also starts calling at a higher intensity. More activity is seen amongst the calling males - mostly jumping from one place to another, but all jumps are confined around and across that female, which responded by giving the reciprocal call.

L. limnocharis and *E. cyanophlyctis* (Plates 2 and 3) females called while submerged in water, making the feeble reciprocal calls very difficult to hear and record and the

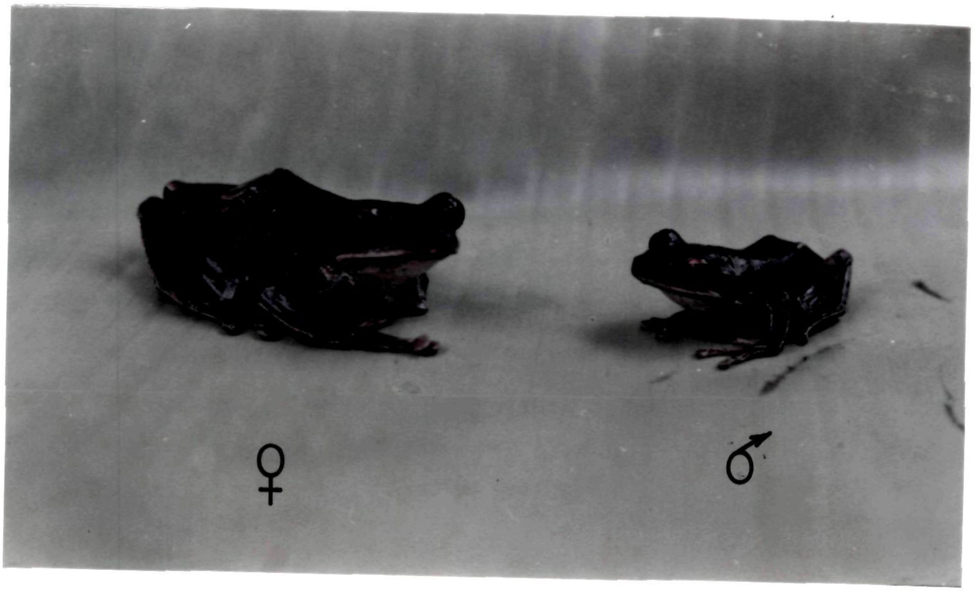
Plate 1.

Female and male of *Polypedates leucomystax.*

Plate 2.

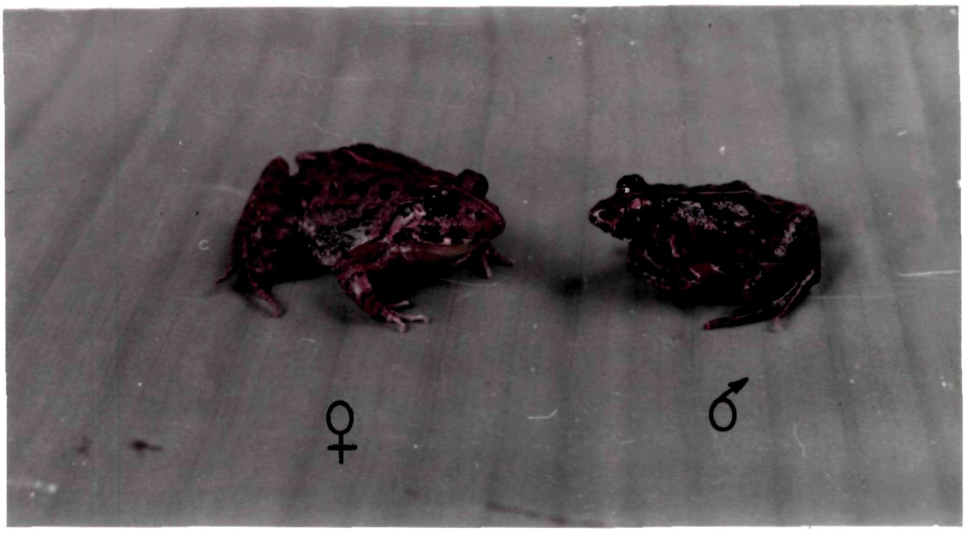
Female and male of *Limnonectes limnocharis.*

PLATE -1



Polypedates leucomystax

PLATE-2

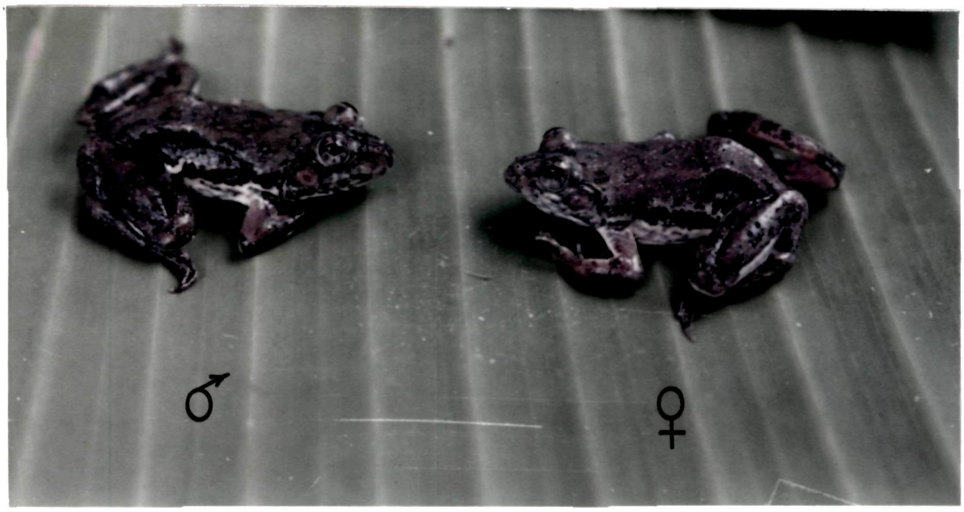


Limnonectes limnocharis

Plate 3.

Male and female of *Euphlyctis cyanophlyctis*.

PLATE- 3



Euphlyctis cyanophyctis

behavioural displays difficult to observe. In both the species the females responded to the male advertisement calls. Once the reciprocal calls were emitted , the male calls were louder and more activity was seen amongst the males which were nearer to the responding female.

The reciprocal call of *P. leucomystax* is low-pitched, lasting approximately 32 ms. The call sometimes has 3 to 4 subunits, each subunit not lasting more than 10 ms. The call is composed of about 22 pulses. The dominant frequency is at about 1048 Hz, with no harmonics, and the frequency domain extends from about 746 to 1229 Hz, the SPL being 77 dB (Table 1; Fig. 1).

In *L. limnocharis* the calls lasted approximately 61 ms, with 45 pulses. The call had a single dominant frequency at about 1531 Hz, lacking harmonics. The frequency domain is comparatively small, extending from about 1300 to 1767 Hz, the SPL being 70 dB (Table 1; Fig. 2).

In *E. cyanophlyctis* the call duration was approximately 20 ms, composed of 20 pulses, having constant interpulse interval. The frequency distribution was bimodal, with dominant frequency at about 740 Hz and its second harmonic at about 1433 Hz. The frequency domain extends from about 496 to 1746 Hz, the SPL being 65 dB (Table 1; Fig. 3).

DISCUSSION

Comparative spectral analysis of female reciprocal calls with male advertisement calls of *P. leucomystax* (Roy 1997) , *L. limnocharis* and *E. cyanophlyctis* shows that female calls are much shorter in duration, with comparatively more pulses, resulting in reduced interpulse

Figure 1.

A i. Male advertisement call of *Polypedates leucomystax*.

ii. Female reciprocal call of *Polypedates leucomystax*.

B i. An enlarged portion of male advertisement call.

ii. An enlarged portion of female reciprocal call.

C i. Corresponding mean spectra and sonagram of male advertisement call.

ii. Corresponding mean spectra and sonagram of female reciprocal call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

Figure-1

POLYPEDATES LEUCOMYSTAX

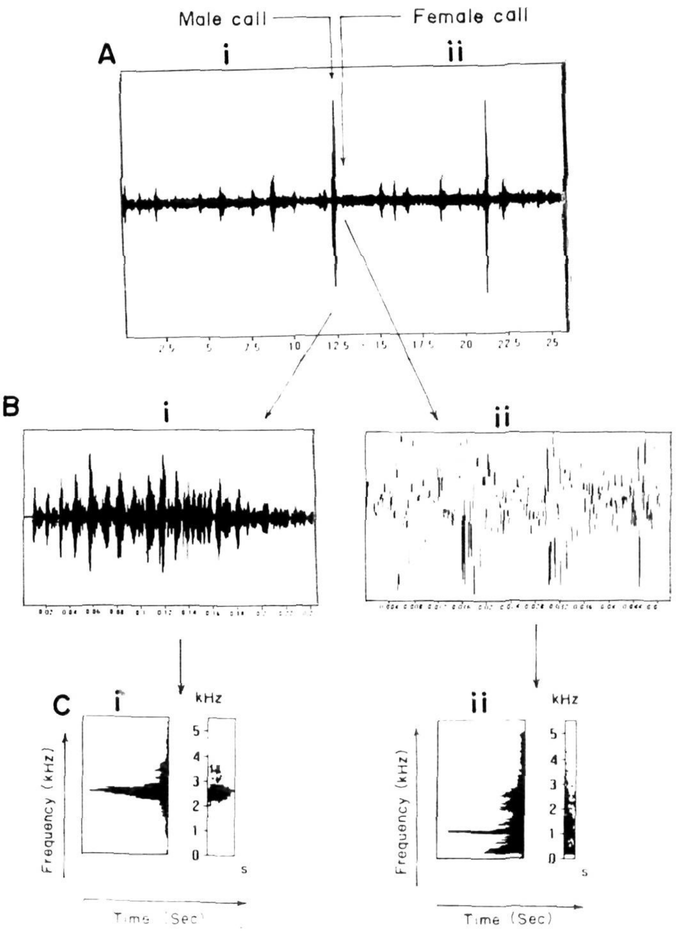


Figure 2.

A i. Male advertisement call of *Limnonectes limnocharis.* .

ii. Female reciprocal call of *Limnonectes limnocharis.*.

B i. An enlarged portion of male advertisement call .

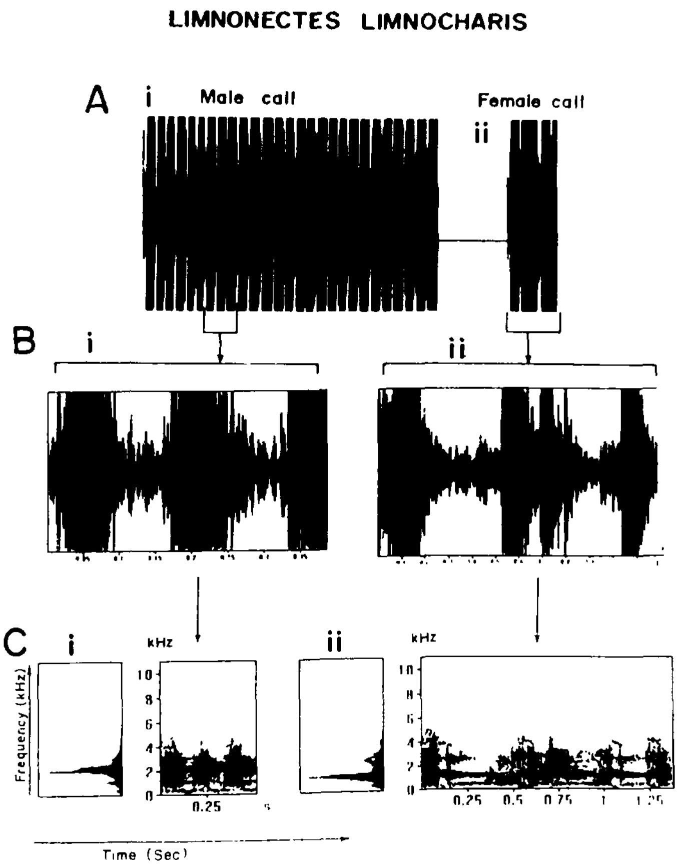
ii. An enlarged portion of female reciprocal call.

C i. Corresponding mean spectra and sonagram of male advertisement call.

ii. Corresponding mean spectra and sonagram of female reciprocal call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

Figure - 2



LIMNONECTES LIMNOCHARIS
A
i
Male call
Female call
ii
B
i
ii
C
i
kHz
ii
kHz
Frequency (kHz)
0.25
0.25
0.5
1
Time (Sec)

Figure 3.

A i. Male advertisement call of *Euphlyctis cyanophlycvtis*.

ii. Female reciprocal call of *Euphlyctis cyanophlyctis*.

B i. An enlarged portion of male advertisement call.

ii. An enlarged portion of female reciprocal call.

C i. Corresponding mean spectra and sonagram of male advertisement call.

ii. Corresponding mean spectra and sonagram of female reciprocal call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

Figure-3

EUPHLYCTIS CYANOPHLYCTIS

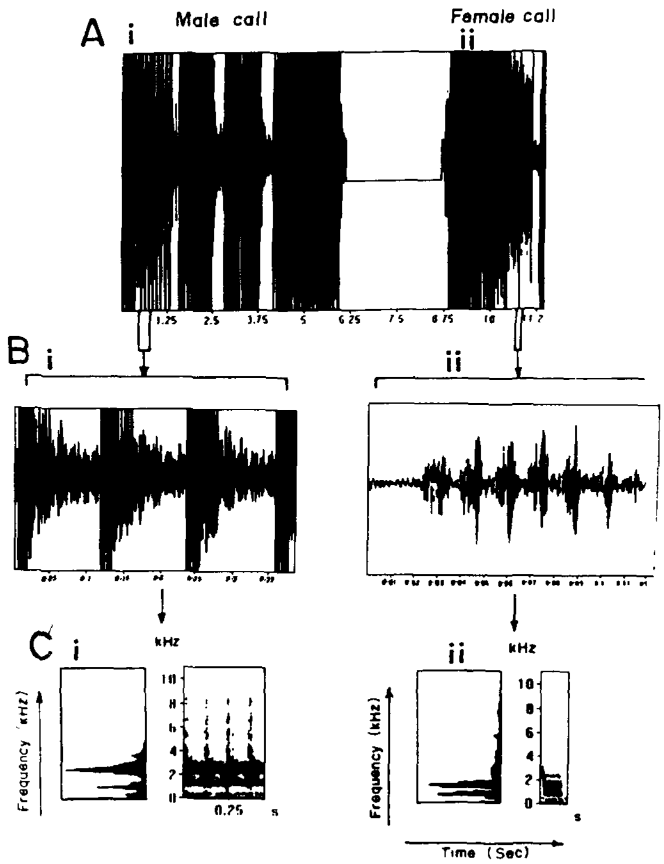


Table 1. Comparative analysis of female reciprocal calls and male advertisement calls of *Polypedates leucomystax, Limnonectes limnocharis* and *Euphlyctis cyanophlyctis.*

					Frequency (Hz)	
Species	**Number of calls per species**	**Call duriation (ms)**	**Pulse number**	**Dominant frequency**	**Lower limit**	**Upper limit**
Polypedates leucomystax						
Female	14	32 ± 9	22 ± 9	1048 ± 105	746 ± 126	1229 ± 114
Male	15	224 ± 4	25 ± 1	2459 ± 42	1495 ± 23	3724 ± 31
Limnonectes limnocharis						
Female	14	61 ± 27	45 ± 7	1531 ± 204	1300 ± 224	1767 ± 213
Male	40	503 ± 101	80 ± 13	2144±1254	1576 ± 87	2685 ± 115
Euphlyctis cyanophlyctis						
Female	12	20 ± 4	20 ± 6	740 ± 36 1433 ± 84	496 ± 57	1746 ± 4
Male	34	615 ± 155	7 ± 1	1651 ± 43 3192 ± 213	1163 ± 124	3907 ± 42

interval and pulse overlap. There is a shift in the frequency domain towards the lower range (almost half) in all the three female reciprocal calls, resulting in a shift of dominant frequency as well. Interestingly, in spite of spectral frequency shift to the lower range, the spectral pattern along with the envelope curve remains unchanged. Like their male counterparts, there is only a single dominant frequency in *P. leucomystax* and *L. limnocharis* and bimodal frequency distribution in *E. cyanophlyctis*.

Female vocalization due to its feebleness and the secretive nature of the females has often escaped the attention of researchers. Most works done till now have been on male vocalization and female phonotaxis experiments. This study has for the first time focused on the role of female vocalization in the breeding biology of the amphibians and demonstrated that the female reciprocal calls seem to act as a ' catalyst ' for the enhancement of the reproductive activity of the breeding colony.

The feebleness of the call may be due to various reasons : (a) The bigger size of the females compared to the males (Blair 1964; Loftus-Hills 1973). (b) The absence of vocal sacs in the females; the expandable pouch-like vocal sac present only in the males acts as a sound resonator and radiator (Duellman and Trueb 1986; Littlejohn 1977; Watkins et. al. 1970). However, little evidence exists to support this hypothesis and it is now being contemplated that the vocal sacs in the males may have a ' variety of mutually nonexclusive fuctions ' ; this is still being worked upon (Dudley and Rand 1991; Rand and Dudley 1993). (c) The differences in the structural morphology and tension of the musculature of the vocal cords and larynx and also the tension of the trunk muscles (Taigen et. al 1985; Marsh and Taigen 1987; Given and Mckay 1990; Given 1993). A comparative study on the morphology as well as the kinematics of the vocal sacs of the conspecific male and female vocal apparatus is necessary to come to a definite conclusion in this matter.

Bioacoustic analysis of amphibian vocalization has emphasized the species-specific temporal and spectral characteristics of the calls. Shift in the temporal pattern occurs which helps avoid acoustic interference and increase the attractiveness of the call is well known (Rand and Ryan 1981; Sullivan and Malmos 1994), but shift in the spectral frequency domain has not been reported. The present study in the three species shows that the frequency domain of the male call is almost double that of the female call and accordingly there is a shift in the dominant frequency, whereas the species-specific spectral pattern of having a single or bimodal frequency distribution remains unaltered. The result of this comparative analysis raises an important question - should the spectral pattern, due to its unaltered species-specific nature, be considered more important than the spectral domain for species identification on the basis of bioacoustic analysis of frog calls ?

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Chapter - 7

INTRODUCTION

The anuran amphibians provide an excellent system for studying animal behaviour. These animals use vocal signals for communication. The vocal signal play a significant role in the social interactions (Wells 1988). While studying acoustic communication in anurans, we came across a very responsive, active and alert species *Polypedates leucomystax* . Its semiarboreal habit makes it a very suitable species for close range visual observation. The vocalisation pattern for both males and females, and their social behaviour was studied for 3 consecutive years - 1993, 1994 and 1995 during their reproductive period from March to August . Wells (1980) has cautioned against about interpreting results from a single year's study of one population as the reproductive activity and their pattern may vary from year to year. This is because amphibians are particularly sensitive to change in rainfall and humidity, exhibiting considerable variability in their social behaviour. He suggested long term studies on tropical frogs to improve understanding the ecology and social organisation of amphibians. Thus present long term study on *P. leucomystax* rules out any such effects of rainfall or humidity.

All studies on the tropical anuran vocalisation and social behaviour is done during the monsoon, which happens to be the breeding period for the animals. During this period, the weather condition is dependent on temperature and humidity. Although it is established that temperature and humidity plays a crucial role in amphibian breeding biology but which out of the two is more effective has not yet been studied. The present study delved into this. The data for the three years showed that on rainy days with humidity above 70%, 4-20 males and

0-4 females were seen in a group. On dry hot day with humidity at 69% or less only 1-10 males and 0-1 females are seen. Similarly the call initiation time is also humidity dependent. The calls are heard approximately 16 min earlier on humid days.

Vocalisation and behaviour of *P. leucomystax* showed many interesting features. The males have graded vocal signals regulated by their proximity (Wells 1980) and distance (Brenowitz et al. 1984) between the calling males. Depending upon the distance, changes occur in the call structure showing distinct behavioural display. More aggression is seen amongst the calling males after the appearance of females (Grobler 1972, Tobias et al. 1998). Although advertisement is generally rare in the amphibian females but in the recent past it has been reported from *Rana virgatipes, Alytes muletensis* and *Xenopus laevis* (Given 1993, Tobias et al. 1998). Amongst Indian species female reciprocal call has been reported in *Limnonectes limnocharis, Euphlyctis cyanophlyctis* and *P. leucomystax* (Roy et al. 1995, Roy 1997). Female reciprocal call acts as a catalyst by enhancing the reproductive activity of the breeding group.

As cited by other workers (Forester and Czarnowsky 1985 , Gerhardt 1987, Klump and Gerhardt 1987, Tobias et al. 1998) in *P. leucomystax* also female calling increases (i) amplitude modulation of the male advertisement call (ii) the length of the call (iii) individual call length , with more activity of the calling males around and across the responding females.

The establishment of the sexual contact in terms of amplexus results after the change from advertisement call of the male to courtship call with close vocal and visual interaction. Each group has a largest and heaviest male, which starts advertising first each evening.

Unlike other calling males of the group the first calling male remains undisturbed and calls persistently throughout the evening without changing its call pattern. The responding female always chooses this first calling male of the group as its mate.

MATERIALS AND METHODS

P. leucomystax is widely distributed in eastern India (Assam, Meghalaya, Mizoram and West Bengal), Burma, Malaysia and Indonesia. These frogs are mostly found perched on creepers (Plate 1A), which entwine bamboo fencing or on tall grass near the vicinity of water. Their perching habit makes them easier to observe than aquatic species and follow their elaborate courtship behaviour.

These frogs can be mistaken for a tree frog due to its climbing habit and the presence of distinct discs at the tips of fingers and toes (Plate 1B). The females of this species are always larger and heavier than males.

Observations were made at a pond measuring approximately 24m x 15m x 2m located inside a private fish farm at Guwahati, Assam (200m ASL; 91.5$'$ E ; 26.1$'$ N) from early March to late August for three consecutive years - 1993, 1994 and 1995 for 4 study groups of frogs. Since the work was initiated in 1993 during mid breeding period, the data for 1993 are less in comparison to 1994 and 1995. Therefore at times for computational purpose, 1993 data are not taken into account. Observations started daily from around 1630 hours and continued-upto midnight. When the frogs called until dawn the observations were continued upto 0430 hours. Torch lights, kerosene lamps, mechanical counters and stop watches were used to locate, count the number of males and females in each group, to record the call

Plate 1 A.

Perching habit of *Polypedates leucomystax*.

Plate 1 B.

Climbing habit of *Polypedates leucomystax*.

PLATE-1

A

B

initiation time, total call time and intercall intervals and social interacions in a group.Weather conditions, temperature and humidity were recorded daily. Frogs were tagged and visual contact was maintained with the calling males and responding females each day throughout the observation period.

The calls were recorded with a unidirectional AKG C451EB shotgun condenser microphone held approximately 60 - 100 cm away from the calling males and 40 - 60 cm away from the calling females. The calls were recorded with a professional SONY WM - 6DC cassette recorder and stored on good quality audiotapes. Sound pressure level was measured by CYGNET 2021 sound pressure level meter held approximately 100 cm away from the calling frog.

Recorded acoustic stimuli were digitized via a Microsoft A/D interface board on to an IBM PC and stored. Oscillograms, sonograms and mean spectra were prepared with a computerised Fast Fourier Transformation analysis (FFT) after passing through band pass filters. The computer analysis provided mean values and standard deviation for call duration, pulse number, dominant frequency and frequency domain. The detailed description of the call parameters is in Roy and Elepfandt (1993).

RESULTS

1. Effects of weather condition on :

A. Appearance of males and females

In course of the study, it became evident that the appearance of males and females is dependent on the weather condition. In order to find the dependence pattern, the data for the appearance of the males and females in the 4 study groups were classified according to the weather condition - rainy, cloudy and sunny for 1994 and 1995 seperately giving their mean and variance (Table 1A). Various test statistics have been computed and values have been tabulated for males and females separately (Tables 1B and 1C). From Tables 1B and 1C it is cleaer that there is a significant difference between average numbers for both males and females appearing on rainy, cloudy and sunny days. The appearance number for both the sexes show similar trend- **sunny > cloudy > rainy**.

Since the appearance of both the sexes were weather dependent, we now wanted to ascertain how the appearance was dependent on the two vital weather parameters - temperature and humidity. Table 1D shows that there exists no correlation between temperature and appearance of males and females. This result is not unexpected as there is not much temperature difference ($\pm$ 4^0 C) during the study period (March-August) at Guwahati.

Having studied the effect of temperature, the effect of humidity was analysed. Table 1E showed that the correlation between humidity and number of male and female appearance was good 0.6 and 0.4 respectively. Both reject the null hypothesis showing population correlation coefficient is 0 at 95% probability level. Hence the appearance is humidity dependent. Two variable regression model on humidity (independent variable) and appearance (the dependent variable) was tested. The fitted models for males was $y = 7.064 + 0.26152x$ and for females $y = - 1.564 + 0.04313x$. In both cases, the slope coefficient is found to be significant at 5% probability level thereby supporting the hypothesis that humidity and number of appearance of males and females are correlated.

Table 1A. Appearance number of males and females according to weather condition.

	1994			1995		
Weather condition	**n**	**Mean**	**Variance**	**n**	**Mean**	**Variance**
MALE						
Rainy	24	11.21	29.90	24	11.08	25.49
Cloudy	24	7.83	17.22	24	6.83	10.39
Sunny	24	5.46	13.58	24	4.29	6.54
FEMALE						
Rainy	24	1.67	2.81	24	1.46	1.75
Cloudy	24	0.83	0.81	24	0.71	0.62
Sunny	24	0.32	0.32	24	0.33	0.31

Table 1B. Tests to determine whether appearance of males is weather dependent.

Comparison between average number of males seen on -	Test applied	Computed value of test statistic
1994		
Rainy and cloudy days	t-test	2.35756
Rainy and sunny days	t-test	4.1769939
Cloudy and sunny days	t-test	2.049378
1995		
Rainy and cloudy days	t-test	3.3999624
Rainy and sunny days	* Between Fischer test	12.168423
Cloudy and sunny days	t-test	2.962573

All tests were significant at 5% probability level for one sided alternative hypothesis.

* Neymann-Bartlett solution of the Behrens Fischer Problem was applied.

Table 1C. Tests to determine whether appearance of females is weather dependent.

Comparison between average number of females seen on -	Test applied	Computed value of test statistic
1994		
Rainy and cloudy days	Behrens Fischer test	4.05
Rainy and sunny days	Behrens Fischer test	4.86
Cloudy and sunny days	t-test	2.074
1995		
Rainy and cloudy days	Behrens Fischer test	4.34
Rainy and sunny days	Behrens Fischer test	5.556
Cloudy and sunny days	t-test	1.866

Table 1D. r values of appearance of males and females for 2 years under different temperatures.

Weather condition			22^0 - 26^0 C	24^0 - 30^0 C	26^0 - 32^0 C
Group 1	1994	M	0.269	0.066	0.023
		F	0.481	-0.056	-0.354
	1995	M	1.080	0.128	0.356
		F	0.395	0.066	-0.161
Group 2	1994	M	0.360	0.110	-0.018
		F	0.356	0.067	-0.351
	1995	M	-1.110	0.142	0.225
		F	0.218	0.411	0.092
Group 3	1994	M	0.342	0.000	-0.014
		F	0.126	0.101	-0.081
	1995	M	0.264	0.081	0.133
		F	0.154	-0.202	0.112
Group 4	1994	M	0.106	-0.126	-0.182
		F	-0.039	0.273	-0.374
	1995	M	-0.037	0.398	-0.176
		F	0.231	-0.337	0.133

Table 1E. Correlation between appearance of males and females and humidity.

	Correlation coefficient	Value of t-statistic	Degree of freedom	5% table value of t distribution
Males	+ 0.61	9.92	166	1.96
Females	+ 0.424	6.03	166	1.96

All tests were significant at 5% probability level.

B. Number of males and females

As humidity is directly correleted with the number of appearance of the frogs in the group, we wanted to test and predict how many males and females will appear depending upon the weather condition . Frequency distribution of males and females are presented in Table 2.

To this observed frequency distribution, we tried to fit an appropriate probability distribution and thereafter determined a confidence interval on the appearance number of *P. leucomystax*. Using chi square goodness fit test, it was found that the negative binomial distribution fitted well. The estimate of parameters of negative binomial distribution fitted to frequency distribution on appearance of males on rainy days was $r = 9$, $p = 0.44285$. Calculated x^2 being 6.1, the null hypothesis of goodness fit was accepted at 5% probability level. By studying the probabilities at $x = 0, 1, 2, \ldots$ it is seen that $p (4 \leq x \leq 20)$ 0.090. Hence we can conclude that the number of males appearing in group on rainy days is likely to be in the range of 4 - 20.

The estimate of parameters of the negative binomial distribution fitted to the frequency distribution on appearance of males on sunny days was $r = 4$ and $p = 0.4687$. Calculated x^2 being 7.12, the null hypothesis of goodness fit was accepted at 5 % probability level. By studying the probabilities at $x = 0, 1, 2\ldots$ it is seen that $p (1 \leq x \leq 10) > 0.90$. Hence we can conclude that the number of males appearing on sunny days is likely to be in the range of 1 - 10.

Similarly the same tests applied to the number of females appeared on rainy days was $r = 3$ and $p = 0.683$. x^2 being 7.29, $p = (x \leq 4) > 0.90$, resulting in 0 - 4 number of females to

Table 2. Frequency distribution of average number of males and females seen in a group per month for 1994 and 1995.

	Males			Females	
	Observed frequency			Observed frequency	
	Rainy	Sunny		Rainy	Sunny
	0	7		16	33
	0	4		9	13
	1	10		13	2
	3	4		3	-
	3	6		4	-
	1	4		3	-
	5	6		-	-
	4	0		-	-
	1	2		-	-
	7	2		-	-
	0	1		-	-
	7	0		-	-
	2	0		-	-
	1	2		-	-
	3	0		-	-
	1	0		-	-
	0	0		-	-
	4	0		-	-
	0	0		-	-
	3	0		-	-
	1	0		-	-
	1	0		-	-
Total frequency	48	48	**Total frequency**	48	48
Mean	11.292	4.875	**Mean**	1.563	0.354
Variance	25.498	10.401	**Variance**	2.288	0.312

appear on rainy days. As regards number of females appearing in group on sunny days, the probability of it exceeding 1 is very small, indicating it is very likely that the number would be 0 or 1.

C. Call time.

Having proved the dependence of the number and appearance of the males and females on humidity, we wanted to find out the effect of humidity on the initiation of the 1st advertisement call timing for a group. To ensure easier analysis, we shifted the origin of call timing to 5.45 P.M. for all recorded data (e.g. if the 1st advertisement call was recorded at 5.30 P.M. , we took it as -15). After shifting all data points, the correlation coefficient between humidity and 1st call initiation time was analysed. The correlation coefficient was high being -0.68. The -ve sign is significant, indicating that the relationship works in opposite direction, higher the humidity earlier the call initiation time.

To investigate this phenomenon in depth, the 115 observation days for 1994 and 1995 were dichotomised as humid when humidity level was 70% or above and non-humid with humidity level at 69% or less. The 1st call timing were grouped accordingly, thereafter the mean and variance of their call initiation time was computed (Table 3). To examine the mean values are significantly different, t - test for testing differences of means was done. Value of test- statistic being 4.48 that is significant at 5% probability level. The average call time for humid days was -8.5 min i.e. on average 1st call on humid days were heard at (5.45 P.M. - 8.5 min =) 5.36 P.M. on the otherhand, the average 1st call time for non-humid days was +7.5 mins i.e. on the average the 1st male call for non-humid days was heard at (5.45 P.M. + 7.5 min =) 5.52 P.M.

Table 3. Statistical measures on 1st call initiation time for a group (with call initiation time at 5.45 pm).

Weather condition in relation to humidity	Number	Mean	Variance
Humid (with humidity $\geq$ 70%)	19	- 1.5789	252.77
Non-humid (with humidity < 70%)	96	13.22917	154.156

A t-test for testing the significance of difference between call times of humid and non-humid days gave a value of 8.5 which is highly significant at 5% probability level (degree of freedom 487). Hence it is evident that the 1st call times do differ between humid and non-humid days. In fact we can accept the one sided alternative hypothesis and conclude that the 1st call time on humid days is significantly earlier than non-humid days. On an average 1st male call is heard about (- 8.5 - 7.5 =) 16 mins earlier on humid than non-humid days. 95% confidence interval of the difference between the 1st call time of humid and non-humid days is 12.5 and 19.5 min.

We also tested the relationship between temperature and 1st call time. The correlation coefficient was only 0.3. A t - test for significance of this observed value rejected the null hypothesis showing the population correlation coefficient to be 0. Hence it is clearly indicated that only a weak relationship exists between temperature and 1st calling time.

2. Vocalisation

The vocalisation is very distinct. The males start advertising very distinctly early in the evening. The female when it decides to respond to the male advertisement, produces a feeble reciprocal call. The male advertisement and female reciprocal calls are very distinct both by listening to the calls as well as looking at their sound spectra (Fig.1).

A. Male vocalisation

Males of this species produce 4 types of calls. The calls are easily distinguishable from each other by the sound, which the animal produces (Fig. 2 ; Table 4).

i. PAK call

The 1st call of the male is always a PAK call, having call duration of 233 ms with 17 pulses, frequency domain extending from 1500 to 3610 Hz with dominant frequency at about 2530 Hz. The sound pressure level being 50.46 dB (Fig. 2Ai and ii).

ii. PAK POK call

Although this call sounds like 1 single unit but has 2 distinct components, each of 213 ms and 112 ms having 10 and 4 pulses respectively separated by 467 ms interval. The frequency domain extend from 860 to 2710 Hz with dominant frequency at about 2500 Hz (Fig. 2Bi and ii).

iii. POK call

These calls are of approximately 150 ms duration with 13 pulses, frequency domain extending from 1020 to 2730 Hz with dominant frequency at about 2090 Hz (Fig. 2Ci and ii).

iv. KOT call

These calls have call duration of 58 ms with 5 pulses, frequency domain extending from 1380 to 2150 Hz having dominant frequency at about 1840 Hz (Fig. 2Di and ii).

B. Female vocalisation

Reciprocal call

The female reciprocal call is given in response to the male advertisement call. This call is low pitched, lasts approximately 32 ms. The call sometimes has 3 to 4 sub units not lasting more than 10 ms. The call is composed of about 22 pulses. Frequency domain extends from 746 to 1229 Hz with dominant frequency at about 1048 Hz. The SPL being 77 dB (Fig. 2Ei and ii).

Figure 1.

A i. Male advertisement call of *Polypedates leucomystax*.

ii. Female reciprocal call of *Polypedates leucomystax*.

B i. An enlarged portion of male advertisement call.

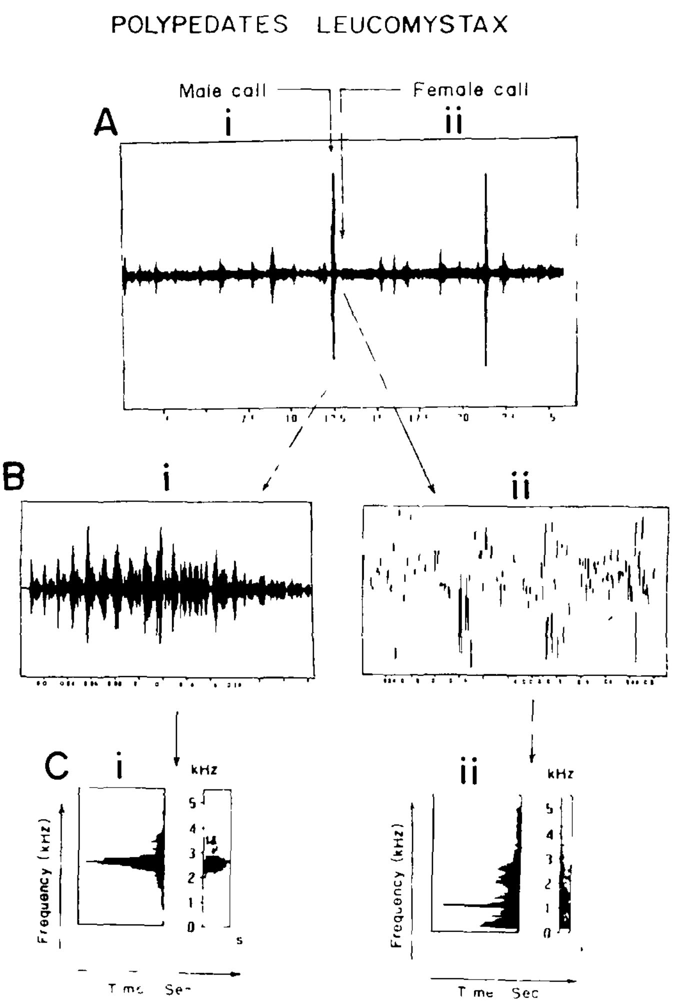
ii. An enlarged portion of female reciprocal call.

C i. Corresponding mean spectra and sonagram of male advertisement call.

ii. Corresponding mean spectra and sonagram of female reciprocal call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

Figure-1



POLYPEDATES LEUCOMYSTAX
Male call
Female call
A
i
ii
B
i
ii
C
i
ii
kHz
Frequency (kHz)
Time Sec

Figure 2.

A i. Oscillogram of the PAK call.

ii. Mean spectrum and sonagram of the PAK call.

B i. Oscillogram of the PAKPOK call.

ii. Mean spectrum and sonagram of the PAKPOK call.

C i. Oscillogram of the POK call.

ii. Mean spectrum and sonagram of the POK call.

D i. Oscillogram of the KOT call.

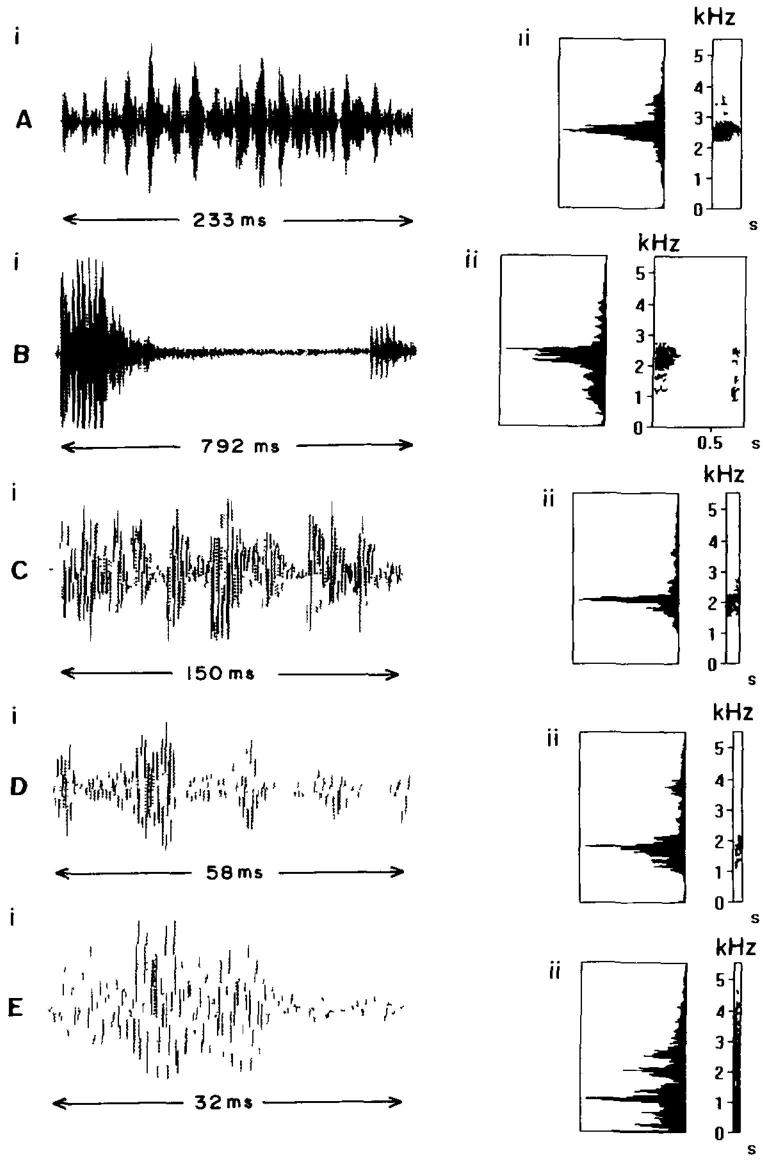
ii. Mean spectrum and sonagram of the KOT call.

E i. Oscillogram of the female reciprocal call.

ii. Mean spectrum and sonagram of the female reciprocal call.

(FFT length - 256; Overlap - 50% ; Windows - Hamming)

Figure-2



POLYPEDATED LEUCOMTX

Table 4. Different call pattern of *Polypedates leucomystax*.

Call pattern	PAK	PAK POK	POK	KOT	Reciprocal
Number of calls	281	175	75	93	85
Call duration					
Mean	233	792	150	58	32
SD	±10	±94	±70	±5	±9
Pulse number					
Mean	17	14	13	5	22
SD	±10	±5	±4	±0	±9
Dominant frequency					
Mean	2530	2500	2090	1840	1048
SD	±40	±25	±20	±10	±105
Lower frequency					
Mean	1500	860	1020	1380	746
SD	±80	±110	±120	±235	±126
Upper frequency					
Mean	3610	2710	2730	2150	1229
SD	±30	±40	±90	±20	±114

3. Call behaviour

A. Male

The males emit their high pitched calls sitting on elevated perches facing towards the centre of the pond . The 1st male call is always a PAK, which is heard around 1730 hours. After about 5 - 10 min the 2nd male call is heard which is also a PAK call. With the passage of time more and more PAK calls are heard from the males of the group who position themselves atleast 60 cm apart. After about 30 - 40 min of the 1st male call and with more males joining the calling, the distance between the calling males which until now was 60 cm starts reducing. With this reduction of intermale distance, some of the males change their PAK call to PAK POK call. Now a mixture of PAK and PAK POK call is heard which do not overlap each other. The 1st PAK calling male continues to call PAK.

After about 1 hour from the time of the 1st male call, the distance between some males producing PAK and PAK POK calls reduces further to less than 30 cm. With the reduction of the intermale distance the two males which were now closest to each other changes their call to POK call. Here also the 1st PAK calling male remains undisturbed by the proximity of the other calling males and continues with its PAK call. While calling POK the frogs come further closer and become aggressive. When the distance between two POK calling males facing each other reduces to about 5 cm they start fighting. When fighting the frogs emit KOT-KOT sound. Once the fight gets over, the frogs move away from each other and continue to call either PAK or PAK POK depending upon the intermale distance. The first PAK calling male neither changes its PAK call nor gets involved in fights.

Calling males located	**Calling males located**	**Calling males located**	**Calling males located**

PAK------------------> PAK -------------> PAK POK ------------> POK ----------------->KOT

atleast 60 cm apart	**between 60 - 30 cm apart**	**less than 30 cm apart**	**about 5 cm apart**

Thus the male vocalisation seems to be regulated by the distance between two calling males during mate attraction . Depending upon the distance between two calling males, changes occur in the call structure having distinct behavioural displays. The calls are distinct from each other can easily be proved by their call structures (Fig. 3 ; Table 4) and call intensities measured in decibels (Tables 5A and B). The values of tables 5A and B can be represented as PAK POK>PAK = POK>KOT.

As has already been described the males in a group start to call one by one. They do not overlap their calls and do not form a chorus. In each colony one particular male start calling. The 1st, 2nd and 3rd male calls can easily be located and identified. There seems to be a perfect time interval between the initiation of the 1st, 2nd and 3rd calling males. For easier analysis and better understanding the origin of time of calling was shifted to 5.45 pm and date wise data for both the years were tabulated.

	Mean	S.D.
1st calling male (PAK)	4.2 min	17.97
2nd calling male (PAK)	11.7 min	20.07
3rd calling male (PAK)	18.6 min	21.19

Figure 3.

Calling sequence and behavioural displays of the advertising male and responding female of *Polypedates leucomystax*.

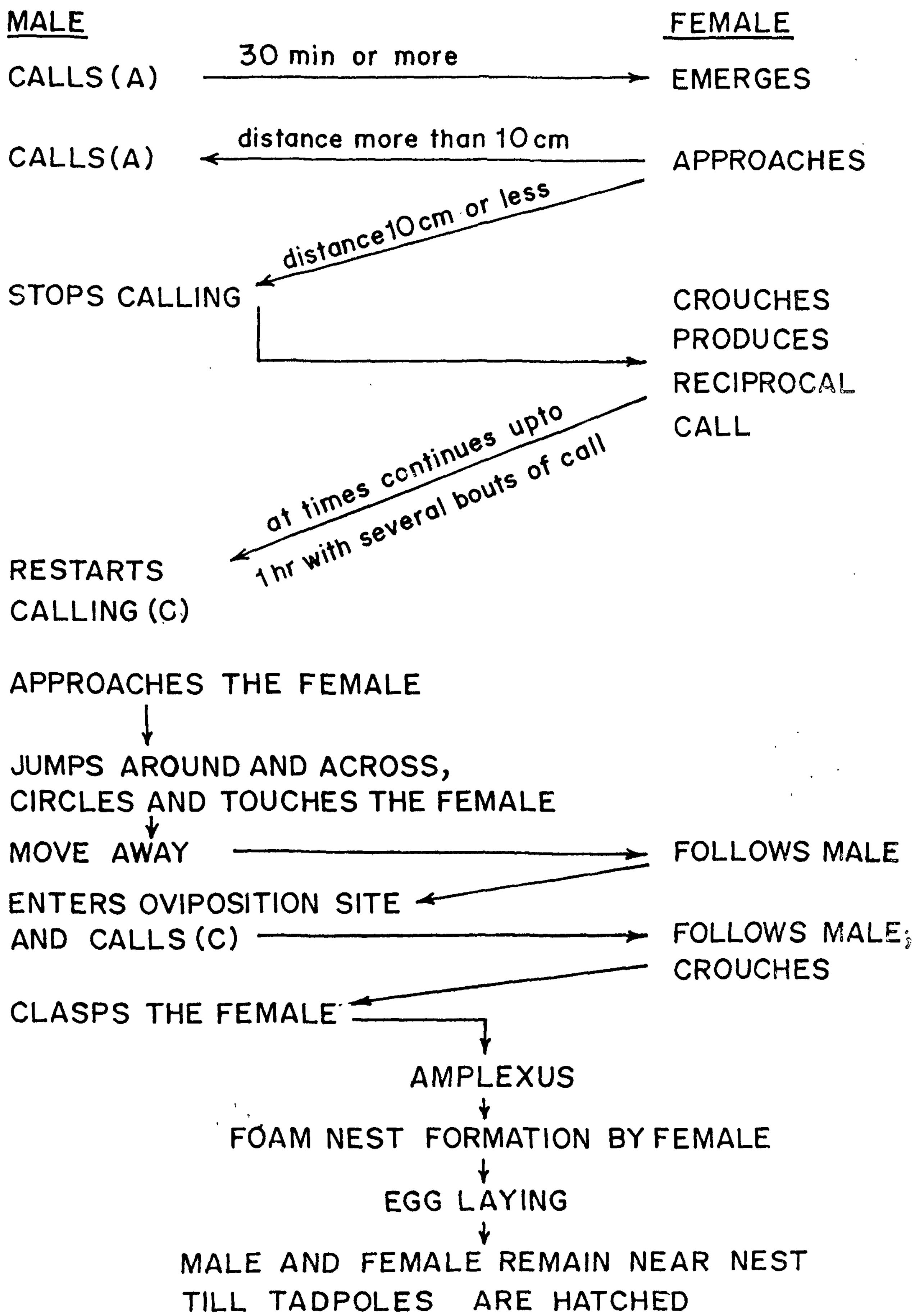

MALE
FEMALE
30 min or more
CALLS (A)
EMERGES
distance more than 10 cm
CALLS (A)
APPROACHES
distance 10 cm or less
STOPS CALLING
CROUCHES
PRODUCES
RECIPROCAL
CALL
at times continues upto
1 hr with several bouts of call
RESTARTS
CALLING (C)
APPROACHES THE FEMALE
JUMPS AROUND AND ACROSS,
CIRCLES AND TOUCHES THE FEMALE
MOVE AWAY
FOLLOWS MALE
ENTERS OVIPOSITION SITE
AND CALLS (C)
FOLLOWS MALE;
CROUCHES
CLASPS THE FEMALE
AMPLEXUS
FOAM NEST FORMATION BY FEMALE
EGG LAYING
MALE AND FEMALE REMAIN NEAR NEST
TILL TADPOLES ARE HATCHED

Table 5A. Call intensities (in dB) of the 4 types of male calls.

Call types	Number	Mean (in dB)	Variance
PAK	13	66.8	5.198
PAK POK	13	72.7	2.045
POK	13	65.9	43.296
KOT	13	53.8	20.885

Table 5B. Comparison of mean intensities of the 4 types of male calls.

Comparison between	Test applied	Value of t-test statistic	Remarks
PAK and PAK POK	t-test	7.58	Significant at 5% probability level for the one sided alternative hypothesis
PAK and POK	Behrens Fischer test	0.4634	Not significant
PAK POK and POK	Behrens Fischer test	3.858	Significant at 5% probability level for the one sided alternative hypothesis
POK and KOT	t-test	2.51	Significant at 5% probability level for the one sided alternative hypothesis

Paired t-test showed significant difference between 1st and 2nd and 2nd and 3rd PAK calls. The value being 63.475 and 40.27 respectively. The degree of freedom for both was 488. Table value being 1.645 (for one side alternative) and null hypothesis was rejected for both. Hence it is evident that 1st and 2nd call times were significantly different and the 2nd call occurs after a significant statistical time interval (11.7 - 4.2 =) 7.5 min. Similarly the time interval between 2nd and 3rd call is (18.6 - 11.7 =) 6.9 min. Correlation coefficient between 1st and 2nd call was 0.99 and 2nd and 3rd being 0.98. Once again the values indicate the existence of a significant time interval between the calls. 2 variable regression analysis on 3 call times can be used to predict the call timing using the following equations :

$$X_2 = 7.1662 + 1.11303\, x_1 \quad \text{................} (\text{i})$$

$$X_3 = 6.4611 + 1.03959\, x_2 \quad \text{.................} (\text{ii})$$

Where X_1 is the 1st call variable, X_2 is the 2nd call variable and X_3 is the 3rd call variable.

B. Female

After the males have advertised by producing PAK and PAK POK calls for about 30 mins, the receptive and responding females emerge mostly from under leaf litter. This female always climb up and positions herself near the first calling male. When the distance between the first PAK calling male and the approaching female reduces to about 10 cm, the male which had been calling till then stop advertising. The female then makes a feeble reciprocal call in response to the male PAK call. This feeble call of the female at times continue upto 1 hr, with several bouts of call. Only after the female responds to the male advertisement call, the male which had stopped calling on the appearance of the female restarts calling as PAK with a higher intensity (Fig. 3).

During 1994 and 1995 this phenomenon of PAK call intensity difference for the same PAK calling male before and after female appearance was observed for 85 days (Table 6). Average for 2 years for PAK call before was 47.916 dB and after was 59.149 dB. The calculated value at 5% probability level was highly significant, 55.2415 for 1995 and 30.53758 for 1994 with 1.645 and 1.76 as degree of freedom respectively. Thus it is clear that the two PAK calls are different both statistically and functionally. Therefore, the PAK call with higher intensity has been designated as the courtship call.

4. Courtship and amplexus

Once the 1st PAK calling male changes its advertisement call to courtship call, all other neighbouring males also start calling at a higher intensity but not as much as the 1st PAK calling male. More activity is seen amongst the calling males-mostly jumping from one place to another, but all jumps are confined around and across that female, which has responded by giving the reciprocal call.

Amplexus occurs between the 1st PAK calling male and the responding female which produces the reciprocal call. The 1st PAK calling male is always the largest and heaviest (SVL : 5.9 cm - 6.5 cm; Weight : 7.1 gm - 8.5 gm) amongst all other calling males in the group (SVL : 3.1 cm - 6.3 cm ; Weight : 3.1 gm - 8.1 gm). To test this phenomenon - the 1st calling male being the largest and heaviest is always selected by the female, SVL and Weight of 1st and 4th subsequent calling males was recorded for 3 years. Their number, mean and variance has been tabulated for their SVL and Weight separately (Tables 7A and B). In both the Tables 7A and B, variance for the 1st calling male is always 0 indicating that it is always

Table 6. Call intensity (in dB) before and after female appearance for 1994 and 1995.

Call intensity	1994			1995		
	n	Mean	Variance	n	Mean	Variance
Before female appearance	15	49.31	1.945156	70	46.61714	0.74856
After female appearance	15	60.7	1.185333	70	58.81714	2.12885
		(Paired t-test)			(Normal test)	

Table 7A. Snout - vent - length (in mm) of 1st 5 calling males.

	1993			1994			1995		
	n	**Mean**	**Variance**	**n**	**Mean**	**Variance**	**n**	**Mean**	**Variance**
1st calling male	18	5.90	0.00	78	6.20	0.00	115	6.50	0.00
2nd calling male	18	5.26	0.19	78	5.89	0.06	115	6.02	0.03
3rd calling male	18	4.99	0.33	78	5.96	0.01	115	6.04	0.02
4th calling male	17	4.82	0.23	78	5.85	0.05	115	6.01	0.02
5th calling male	15	4.78	0.22	78	5.72	0.15	115	5.83	0.15

Table 7B. Weight (in gm) of 1st 5 calling males.

	1993			1994			1995		
	n	**Mean**	**Variance**	**n**	**Mean**	**Variance**	**n**	**Mean**	**Variance**
1st calling male	18	7.00	0.00	78	8.00	0.00	115	8.50	0.00
2nd calling male	18	5.11	0.58	78	7.05	0.37	115	7.47	0.30
3rd calling male	18	4.72	0.65	78	7.21	0.08	115	7.50	0.19
4th calling male	17	4.47	0.25	78	6.87	0.40	115	7.41	0.23
5th calling male	15	4.47	0.25	78	6.47	0.84	115	6.79	0.92

a particular male which begins to call 1st in the evening in the group and is selected by the female. Again the 1st calling male is also the largest and heaviest in comparison to other calling males of the group. It is this 1st calling male which is always choosen by the female for courtship and amplexus (Tables 8A and B).

5. Nest building and egg laying

Approximately 22 hours after the initiation of the amplexus, the female secretes a foamy substance and within 45 min a foam nest is built. The foam nest remains attached to the leaves or tree twigs, which hang directly above the water surface. Once the nest is formed the amplexing pair enters the nest and remains inside for almost 1 hour and eggs are laid. Freshly laid eggs could be counted only on two occasions, which numbered 60 and 120. Once the eggs are laid, the male emerges out of the nest first. Both the males and females are found to guard the nest, the female remaining closer to the nest within a distance of 10 cm.

The eggs are hatched by the third day and the hatchlings emerge as glassy, transparent tadpoles. The tadpoles when hatched fall directly into the pond water.

Maximum number of amplexing pairs and nests are found during late April to early May. The number declines with the passage of time, with almost none in August.

DISCUSSION

Three years study on *P. leucomystax* showed that as in case of higher animals these amphibians too have a well organised social structure. This structure is easily observed during the breeding season when their vocal communication regulates reproductive activities leading to courtship, amplexus, nest building and egg laying.

Table 8A. Testing significance of difference between Snout-vent-length (in mm) of 1st 5 calling males.

	Comparison between mean SVL of calling male	1st and 2nd calling male	1st and 3rd calling male	1st and 4th calling male	1st and 5th calling male
	Calculated t-statistic	6.04	6.45	9.23	9.87
1993	Degree of freedom	34	34	34	34
	5% probability level	1.60	1.60	1.60	1.60
	Calculated t-statistic	11.04	28.84	13.31	10.77
1994	Degree of freedom	154	154	154	154
	5% probability level	1.65	1.65	1.65	1.65
	Calculated t-statistic	30.97	38.64	37.69	18.13
1995	Degree of freedom	228	228	228	228
	5% probability level	1.65	1.65	1.65	1.65

Table 8B. Testing significance of difference between weight of 1st 5 calling males.

	Comparison between mean weight of calling male	1st and 2nd calling male	1st and 3rd calling male	1st and 4th calling male	1st and 5th calling male
1993	Calculated t-statistic	10.57	11.69	20.88	20.88
	Degree of freedom	34	34	33	31
	5% probability level	1.60	1.60	1.60	1.60
1994	Calculated t-statistic	13.77	24.73	15.71	14.64
	Degree of freedom	154	154	154	154
	5% probability level	1.65	1.65	1.65	1.65
1995	Calculated t-statistic	20.65	24.46	24.31	19.11
	Degree of freedom	228	228	228	228
	5% probability level	1.65	1.65	1.65	1.65

As in case of *Colotethus inguinalis* where males have graded vocal signals regulated by the proximity (Wells 1980) and distance in case of spring peepers (Brenowitz et al. 1984) between two calling males, *P. leucomystax* also shows similar interactions. The distance between the two calling males during mate attraction mainly regulates vocal signals in this species. Depending upon the distance, the changes occur in the call structure having distinct behavioural displays. The PAK call seems to be for advertisement, PAKPOK to signal advertisement and function as a territorial call as well, POK as the aggressive call and KOT can be classified as graded aggressive signal (Wells 1989; Gerhardt and Schwartz 1994) produced by the males who feel encroached upon within their own territories by the calling male entrants. More POK and KOT calls are heard as the level of aggression become intense especially when the females appear (Grobler 1972; Tobias et al. 1998).

Female receptive calls and courtship duets are rare in vertebrates and have seldom been reported in amphibians (Given 1993; Tobias et al. 1998). The low pitched feeble reciprocal calls have also been reported from India (Roy et al. 1995; Roy 1997). This led to two very significant findings. Firstly, the female reciprocal call seems to acts as a " catalyst" for the enhacement of reproductive activity of the breeding group. Secondly, till now the general opinion was that females typically initiate sexual contact by moving close to or touching a calling male (Blair 1960; Wells 1980) and the males indiscriminately try to mate with other frogs that move near or touch them (Mecham 1961; Gerhardt 1974). The present findings clearly indicate that prior to sexual contact the following should happen sequentially: (i) distance between the advertising male and the responding female has to be 10 cm or less ;

(ii) the advertising male which had been calling till then should stop advertising ; (iii) the responding females has to respond by producing reciprocal calls ; (iv) the male, which had stopped calling, should restart calling with a higher intensity - only then sexual contact is established. The establishment of sexual contact in terms of amplexus results after change from the advertising to courtship female and definite moves by both the advertising male and the responding female which appear to be distance-dependent. Thus a close vocal and visual interaction underlies courtship behaviour in *P. leucomystax.*

Another interesting observation has been the preference of the female always for the first PAK calling male. It is logical that males, which outsignal competitors, enhance the probability of their being noticed by sexually active females (Parker 1983). This might be accomplished by employing any of the following strategies - (a) increase in the length of individual calls (Klump and Gerhardt 1987) ; (b) increase in the intensity of the call (Forester and Czarnowsky 1985; Gerhardt 1987) ; (c) persistant calling throughout the course of the evening (Forester et al. 1989). The males that employ any of these strategies, regardless of their size, increase the chances of being selected by conspicuous contain more acoustical energy are indicative of good physical condition. Females, which respond to such call , should benefit more than females mating randomly (Klump and Gerhardt 1987).

In case of *P. leucomystax* the first PAK calling male does not change its call pattern throughout the evenung. It continues to call PAK and continues persistently. It has been shown that the intensity of the PAK call is less than PAK POK call. The 1st calling male saves its energy initially by calling at a lower intensity. It only starts calling at a higher intensity after the female reciprocal call. This male therefore seems to employ all three strategies and does not seem to be threatened by other calling males, since it does not change its call pattern. This is an indication of its dominant position in the group over all other potential competitors. Thus such a male which has conspicuous calls having more acoustical energy, which it stores initially by calling at a low intensity, could be indicative of good physical condition and thus preferred by the females.

Darwin (1871) predicted that larger, older males monopolise mating. Wilbur, Rubenstein and Fairchild (1978) suggested that females choose the largest male available because there is a selective advantage in large body size as it is an indicator of high quality of genotype either because they are older and have demonstrated their survival value or because it is indicative of a fast growth rate. Females could choose males with genes for faster growth rate. In case of P. leucomystax also, the females are always seen to choose the 1st calling male which is the largest and heaviest amongst all other calling males of the group. Weather condition (humidity) has a correlation with appearance of both males and females. More numbers 4-20 males and 0-4 females to be seen on rainy humid days, whereas 1- 10 males and 0 or 1 females on dry non-humid days. Similarly, humidity also effects the initiation of the calling time. Higher the humidity, earlier the call initiation times. On an average the 1st male call is heard about 16 mins earlier on humid days is comparison to non-humid days. Effect of temperature both on number of appearance of the males and females and call initiation time is found to have weak correlation.

Most nest building and egg-laying activity is seen in April, which declines gradually with no nests to be seen from August onwards. This is probably because the animals avoid the wettest season of northeast India (June - August) when there is a risk of the eggs and small tadpoles being washed away by heavy rains. Duellman (1970) has made similar observations for Central American stream breeding hylids and by McDiarmid (1978) in *Centronella.*

Thus it can be concluded that as in the case of higher animals, in amphibians too, there exists a very distinct social structure where acoustic communication plays a key role in formulating this structure.

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