STUDIES ON CERTAIN ASPECTS OF ECOLOGY LOPMENT AND EXPERIMENTAL BREEDING OF *Rana cyanophlyctis* SCHNEIDER

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ABSTRACT

OF

THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

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SHILLONG, INDIA

ABSTRACT

The thesis embodies an investigation on certain aspects of ecology, development and experimental breeding of *Rana cyanophlyctis* Schneider, a common aquatic frog species available at plains and hills of North Eastern India, arranged in six chapters.

Chapter 1 :

Chapter 1 deals with a description of the study sites: (1) Government Fish Farm, Ulubari, Gauhati, Assam (49.4 m a.s.l., 25.05°N and 91.45°E) and (2) Pologround adjoining Umkhrah river, Shillong, Meghalaya (1428 m a.s.l. 25.34°N and 91.56°E). The two sites were selected to find out, whether the frog species under investigation shows any intraspecific or ecological variations or not. The Gauhati site is a properly managed fish farm by the State Government of Assam and represents typical ecological conditions of plains. The Umkhrah river site at Shillong is a natural site surrounded by hills and coniferous trees. The major difference is that the Gauhati site has lotic

water system with alkaline pH and Shillong site has lentic water system with acidic pH. Gauhati has tropical climate and Shillong sub-tropical.

Chapter 2 :

Chapter 2 deals with an analysis of morphological characters, morphometric measurements, length-weight relationship and annual breeding cycle of Rana cyanophlyctis at the two sites. This species is identified by a depressed head, somewhat broader than long, equal size 1st and 2nd fingers, slightly dilated and completely webbed toes and warty skin with pores and tubercles. Males are smaller and lighter (SVL 4.0 - 5.30 cm) and body weight 7.0 - 19.0 gm) and females are larger and heavier (SVL 5.10 - 7.20 cm and body weight 22.0 - 38.5 gm). There was no significant variation in their morphology or morphometry. Linear relationship was obtained between their length and weight. Relative conditions of the frog did not show much variations in different months. The annual breeding cycle is divisible into 3 phases (i) Pre breeding (February to April) (ii) Breeding (May to September (iii) Post breeding (October to January). In winter, they do not hibernate, presumably because of aquatic adaptation, and are often seen basking in morning hours at the edges of water bodies. Statistical analysis does not show any racial difference between two populations.

Chapter 3 :

Chapter 3 deals with relative abundance and homes range movements of *Rana cyanophlyctis* and relationship of these with environmental conditions at both sites. The investigations were made with the help of two techniques, 1) Number catch per unit time and 2) Capture, marked, release and recapture technique. The second technique was attempted at Gauhati only. During winter months (November-February) they remain restricted to the middle core of water bodies except juveniles which are seen on embankments also. With the increase in temperature from March onwards they became more active and their relative abundance on land increases. They maintain a 'home range' which is largest during summer months.

Chapter 4 :

Chapter 4 deals with food and feeding habits of *Rana cyanophlyctis*. The percentage composition analysis reveals that insects formed a very large bulk of the food items of this species at both sites. Homoterans formed highest percentage in Gauhati frogs and Insect larvae and Dipterans formed highest percentage in Shillong frogs. The amount of food intake did not vary much during different phases of annual breeding cycle. The food types reflected food abundance in the ecosystem and their availability in relation to changing environmental conditions. The relationship between length and weight of the alimentary canal and gastro somatic index did not show much variation. Gastro somatic index did not show any significant correlation (P 0.05) with length and weight of the frog also.

Chapter 5 :

Chapter 5 deals with a normal table of development of *Rana cyanophlyctis* investigated after induced breeding. at room temperature 17°C - 21°C in the months of June, July and August. Hatching occurs on 4th/5th day and metamorphosis is completed on 68th day. Entire development has been divided into 40 stages. Fertilization stages 1 - 2, cleavage: stages 3 - 9, gastrulation: stages 10 - 12, neurulation: stages 12 - 18, organogenetic stages 18 - 24, and metamorphic stages 24 - 40.

Chapter 6 :

Chapter 6 deals with induced breeding and gonado-somatic index. Homoplastic pituitaries preserved in absolute ethyl alcohol were used for induced breeding. The pituitaries were as effective even 3 years after their fixation as when they were freshly preserved. The induced breeding was possible for 8 months from March to October. Pituitary dosage 0.08 mg/gm weight of the female induced successful ovulation in females having SVL 5.8 cm and above and weight 25.0 gm and above during breeding period. During pre and post breeding periods larger dosage (0.15 - 0.2 mg/gm weight of female) were required. A linear regression was obtained between SVL and number of ova, present in ovary; SVL and weight of ova; body weight and size of ova; gonadosomatic index and body weight, gonadosomatic index and SVL and gonadosomatic index and weight of ovaries.

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THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENT OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

To

THE NORTH-EASTERN HILL UNIVERSITY
SHILLONG, INDIA

MARCH - 1982

Grams : NEHU

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Dr. M. K. Khare
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8th April, 1982

CERTIFICATE

I certify that this thesis entitled "Studies on certain aspects of ecology, development and experimental breeding of *Rana cyanophlyctis* Schneider" submitted by Mr. Ajai Kumar for the Ph.D. degree of the North-Eastern Hill University, Shillong, embodies the record of original investigation carried out by him under my supervision since 1977. He has been duly registered and the thesis presented is worthy of being considered for the award of the Ph.D. degree. This work has not been submitted for any degree to any other university.

(M.K.KHARE)

D E D I C A T E D

T O

M Y P A R E N T S

CONTENTS

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Principal and Management M.G. Degree College, Gorakhpur

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31st March 1982

Ajai Kumar
Ajai Kumar

General Introduction

The first animals to emerge from the aquatic environment and crawl on land approximate 3 x 10^9 years ago were the ancentral amphibians (Cochran, 1968). Noble (1931) commented that the amphibians were the first vertebrates to develop voice and break silence on this earth. Cinery (1976) describes that the amphibians arose from their crossopterigian ancestors during carboniferous and permian periods and have diversified into a number of species. Of these, 2,600 species belonging to 245 genera and 18 families have till now been reported from different parts of the world.

The value of anurans in human welfare is known since times immemorial. They are considered auspicious items in certain human communities. Certain tribes in Africa tie femur bone of frogs to attain supernatural powers and goodluck. Chinese use toad skin and its poison as therapeutic agent for various heart ailments. Sometime back in an Indian village about 30 km from Mehsameend in drought hit Raipur district of Madhya Pradesh a strange marriage of toad bride and bridegroom was performed with all pomp and show under a special 'mandup' to please Indra, the god of rains (See Amrit Bazaar Patrika dated 25.10.1979).

Apart from such beliefs, frogs and toads enjoy immense economic and academic values. Handerson (1864) reported the sale of dried frogs and toads in China, Japan and South-East Asia for food and medical purposes. He also mentioned their use for preparing fine leather. Frogs and toads have been used as biological control agents to control paddy pests in China and Japan (Okado, 1927) and sugarcane pestsin South America (Pack, 1927; Cochran, 1968). In recent years, their economic value has tremendously increased as frog legs are used as gourmet food items in Japan, France, U.K. and U.S.A. Tons of frozen frog legs worth crores of rupees are exported every year from this country through Marine Products Export Development Authority (MPEDA), Government of India, Cochin. Though the processing of frog legs was originally known to certain communities of adivasis, Bohri community of Gujarat and certain muslim communities of Kerala, it is now scientifically done under hygienic conditions under the supervision of the experts of MPEDA.

Many significant concepts in the field of Developmental Biology, Animal Physiology and Medical Sciences have come up as a result of experimentation of frogs. To mention a few - as quoted by Balinsky (1957), Galvani (1789), while working on exposed nerve and muscle stimulations of dissected frog legs, got an idea that led to the discovery of electric current. Before the availability of sophisticated techniques, frogs and toads were used for pregnancy tests in earliest stages of conception (Shapiro and Ziwarenstain, 1933). Phenomena of differentiation and cloning among vertebrates was first investigated in frogs and toads. Frogs and toads are used freely in schools, colleges and research laboratories for demonstration of vertebrate anatomy and basic experiments in physiology and embryology.

Supply of frogs and toads for all these purposes comes from nature. Indiscriminate collection of frogs and toads from their natural habitats is resulting in rapid depletion of their populations; and this is a threat to their very survival. As such, necessity is being increasingly felt to culture frogs on commercial scale. Priddy and Culley (1971) reviewed the work on frog culture. Attempts on frog culture have been made in U.S.A., Japan, Italy, Austria and England. In U.S.A. 'amphibian facilities' have been developed and varieties of frogs are cultured in closed set up under conditions of controlled temperature, humidity and selected feeding. Keeping in view the high costs of such programme, economic conditions and difficulties due to interrupted electric power supply (due to power shortage) in our country, it may not be feasible to develop technology for frog culture in closed set up. We need a technology which may be practised in ponds in open environment and may be acceptable to farmers.

Attempts have been made in our country by Central Inland Fisheries Research Institute, Barrackpore, West Bengal, at their Kalyani and Cuttack units. Though results are encouraging, we are still far from having evolved reliable methods of frogs culture. It needs hardly be stated that for any attempt in respect of any species, a knowledge of its biology and ecology must be known in detail.

<u>Rana cyanophlyctis</u> Schneider, the skipper frog, is a common aquatic frog species available throughout Indian subcontinent (Satyamurti, 1967). It is also a common frog species available at plains and hills of North-Eastern India. Though systematics, biology and functional morphology of this species has been investigated by certain workers (Boulenger, 1920; McCann, 1933), its ecology, food and feeding habits and breeding behaviour have to be properly understood in a particular biotope to evolve its culture technique in that region. It was with this background that the present investigation was carried out.

Two study sites were selected, one at the plains of Assam and the other at the hills of Meghalaya to investigate the nature of variations in the populations of *Rana cyanophlyctis* available at the two regions. The thesis has been divided into six chapters dealing with (1) description of study sites, (2) diagnostic characters, morphometry and annual breeding cycle, (3) relative abundance, home range and movement, (4) food and feeding habits, (5) normal table of development and (6) experimental breeding. It is hoped that the study will be helpful in maintenance and for undertaking culture programme for this species.

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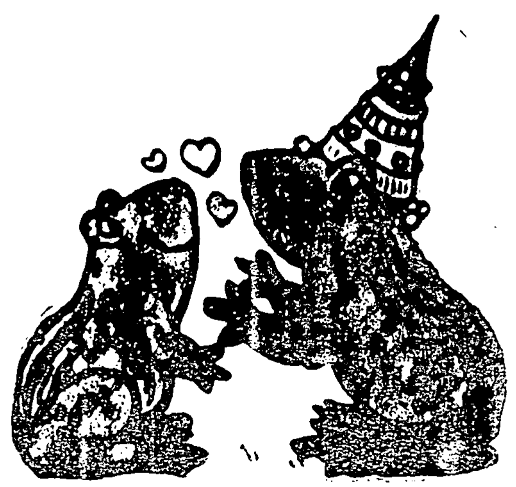
Shapero, H.A. and H. Zwarestain. 1934. A rapid test for pregnancy on Xenopus laevis. Nature Lond. 133: 339-62.

* Original not seen.

CHAPTER-1

STUDY SITES



Rana cyanophlyctis has wide distribution (Boulenger, 1920; Satyamurti, 1967; Daniel, 1975) and is abundantly available on the plains of Assam and hills of Meghalaya. Thus, the work has been carried out at two selected sites - one at Gauhati (Assam) and the other at Shillong (Meghalaya). Details of these sites are given below.

1.0 State Government Fish Farm, Ulubari, Gauhati (Assam)
(49.4 metre above sea level (m.a.s.l.), 26.05°N and 91.45°E) (Fig. 1.1.1)

The Ulubari fish farm is situated about 1½ km, south-east to the Gauhati Railway Station. It was constructed for fish culture and research by the State Fishery Department Assam, in 1953, on the southern bank of Bharlu river, a tributary of Brahmaputra flowing from east to west. The water of river remains muddy and foul due to heavy silting and effluents received from Gauhati Oil Refinary. It has low water level, but during rainy season the water level increases tremendously, though it never overflows the bank of the river. In the west neighbourhood of the farm there are agriculture and food preservation departments. The fish farm is surrounded on three sides, East, West and South, by low lying dry area, which get filled up with 0.5 - 1.0 metre deep water during rainy season. The fish farm has a total area of 19,375 sq metres. It has 17 tanks of different sizes (20 x 18 metres to 90 x 65 metres). The intervening land area of the farm

Fig. 1.1: Map of North-Eastern Region showing study sites.

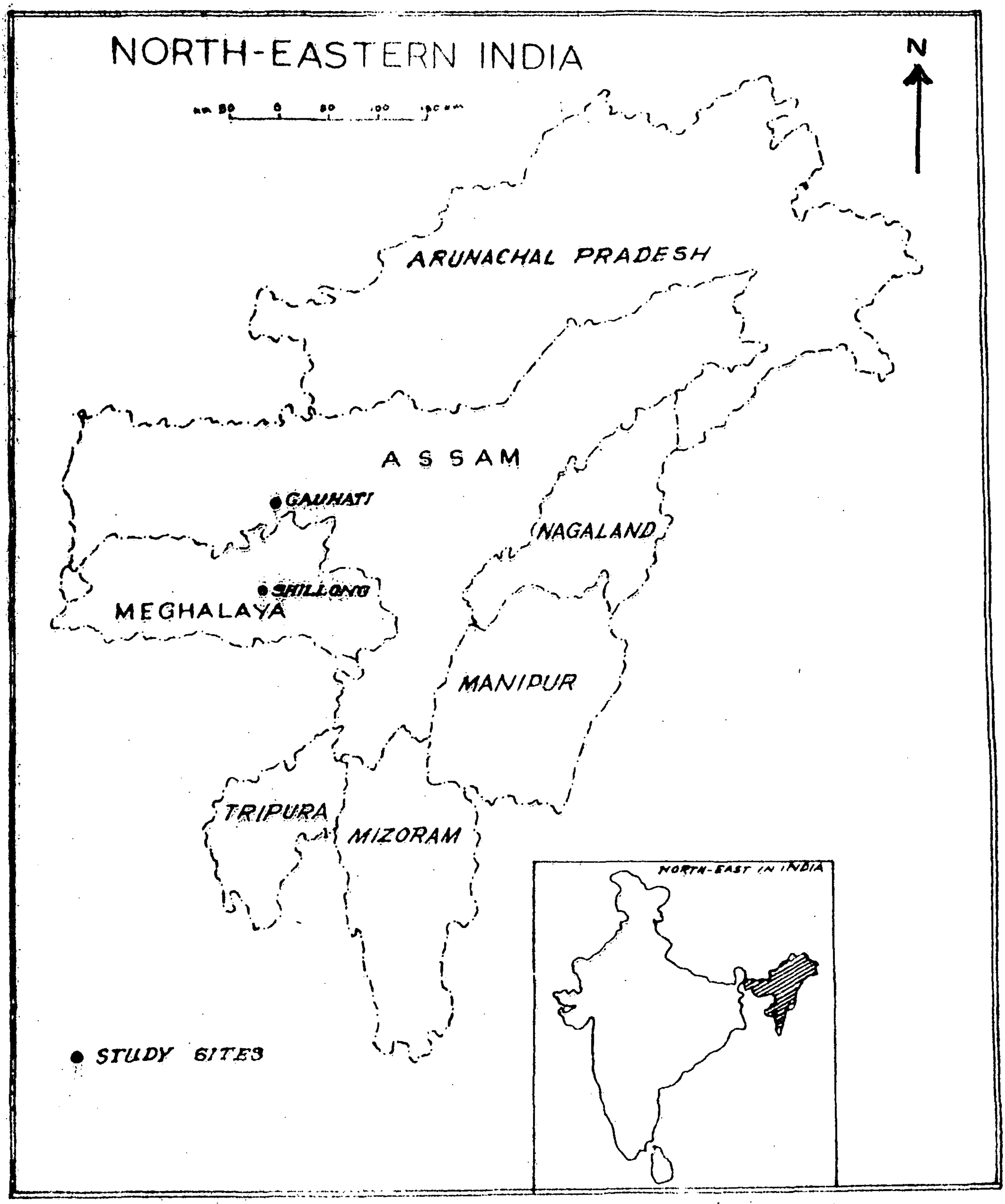

NORTH-EASTERN INDIA
N
ARUNACHAL PRADESH
ASSAM
GAUHATI
NAGALAND
SHILLONG
MEGHALAYA
MANIPUR
TRIPURA
MIZORAM
STUDY SITES
NORTH-EAST IN INDIA

FIG. 1.1

Fig. 1.1.1: Map of the study site, State Fish Farm, Ulubari, Gauhati, Assam.

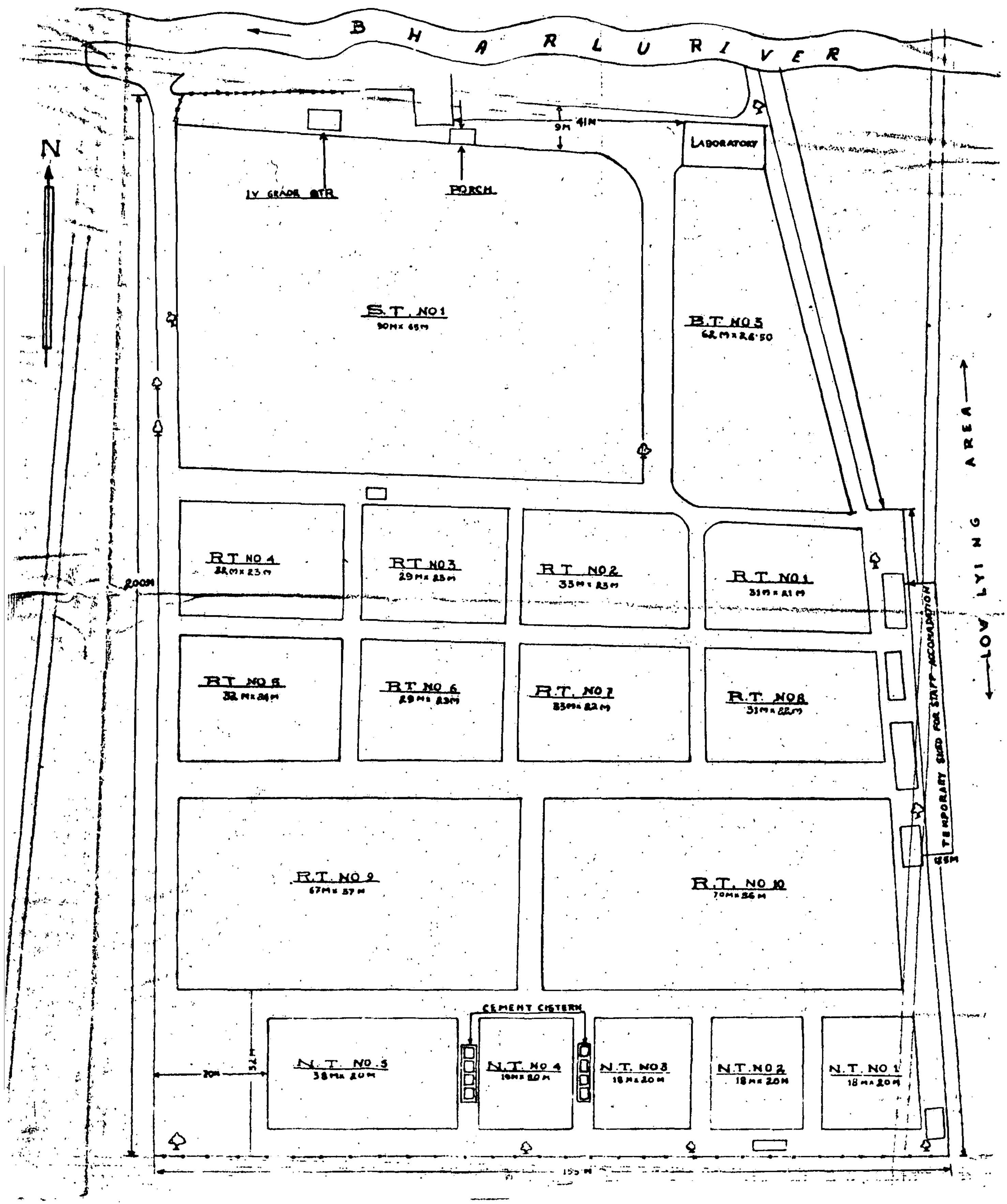

BHARLU RIVER
N
LABORATORY
IV GRADE QTR
PORCH
9M
41M
S.T. NO 1
90M x 65M
B.T. NO 5
62M x 26.50
R.T. NO 4
R.T. NO 3
29M x 23M
R.T. NO 2
33M x 23M
R.T. NO 1
R.T. NO 5
R.T. NO 6
R.T. NO 7
R.T. NO 8
R.T. NO 9
67M x 57M
R.T. NO 10
70M x 56M
CEMENT CISTERN
N.T. NO 5
38M x 20M
N.T. NO 4
N.T. NO 3
18M x 20M
N.T. NO 2
18M x 20M
N.T. NO 1
18M x 20M
200M
20M
32M
155M
TEMPORARY SHED FOR STAFF ACCOMADATION
LOW LYING AREA

Fig. 1.1.1

Fig. 1.1.2 & 1.1.3 : A view of the State Fish Farm, Ulubari, Gauhati, Assam, Study site No. 1.

Fig. 1.22

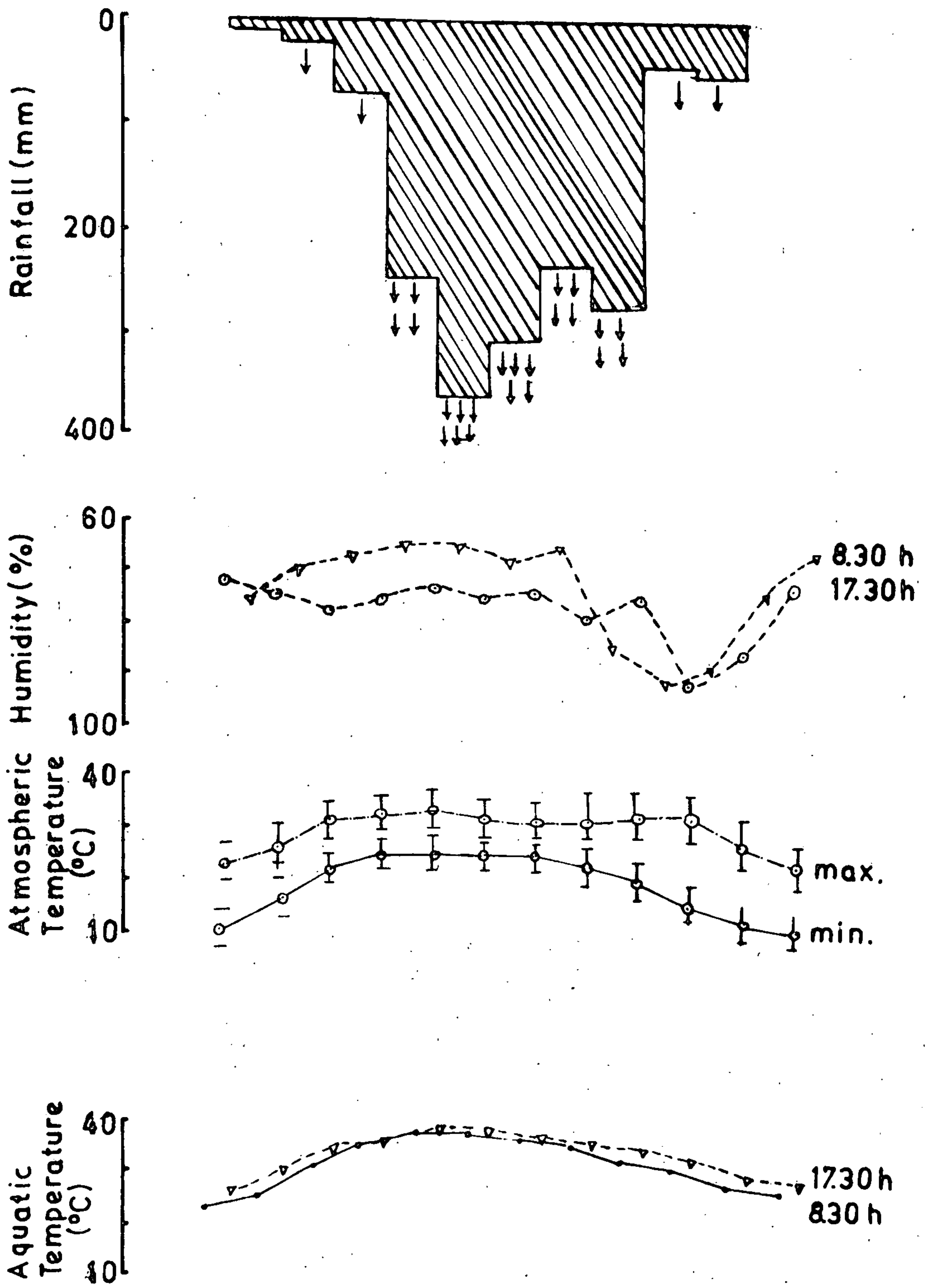
Fig. 1.23

covers 6296 sq metres. At the north-east corner of the farm there is one small laboratory, one pukka water tank and some temporary hutments. At the northern side there are some residential houses for Assam State Government employees (Figs. 1.1.2 and 1.1.3).

The location of Gauhati and so the fish farm is at the tropic of cancer. Gauhati is surrounded on three sides by hills and experiences south-east tropical monsoon from April to October. The beginning of the rains is marked by strong winds, overcast skies, occasional thunderstorm and hail storm during Apil-May. The winter season also experiences occasional showers. The atmospheric and water temperature, rainfall and humidity have been illustrated in Fig. 1.1.4 for 1978. The maximum rainfall, about 375 mm per month, was recorded during May and June and minimum during January, February, March, November and December. Maximum atmospheric and water temperatures were recorded during July and August and minimum during January. The annual humidity fluctuated between 44% to 100% being maximum during June and July. High humidity is the cause of sweating one experiences for a large period of the year. Correlations between different climatic factors have been shown in Table 1.1.1.

The ponds had following flora: algae belonging to families Chlorophyceae, Euglenophyceae, Bacillariophyceae,

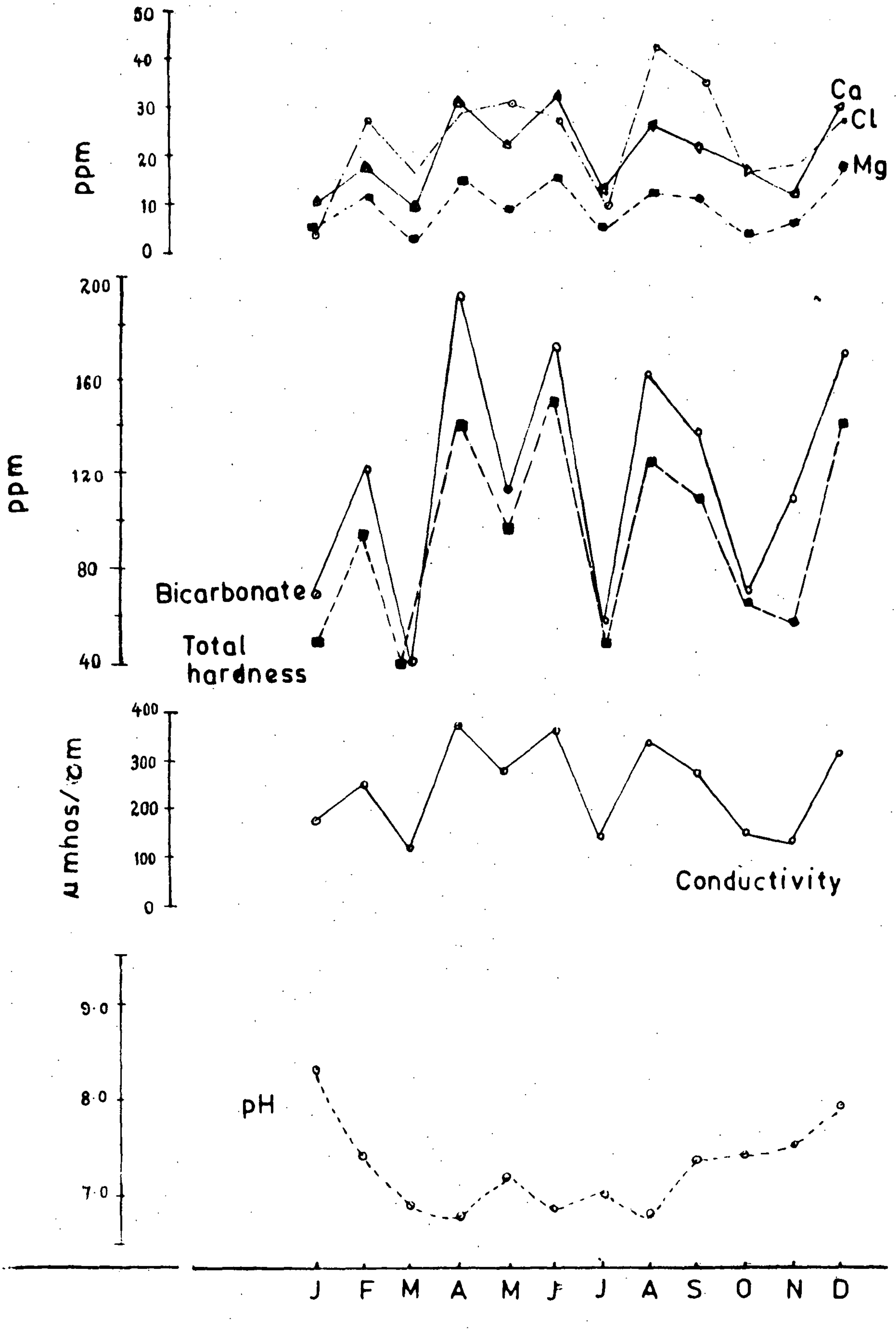
Fig. 1.1.4 : Environmental conditions at Study Site No. during 1978.



0
200
400
Rainfall (mm)
60
100
Humidity (%)
8.30 h
17.30h
40
10
Atmospheric Temperature (°C)
max.
min.
40
10
Aquatic Temperature (°C)
17.30 h
8.30 h

Fig. 1.1.4

Fig. 1.1.5 : Limnological conditions at the Study Site No. I during 1978.



50
40
30
20
10
0
ppm
Ca
Cl
Mg
200
160
120
80
40
ppm
Bicarbonate
Total hardness
400
300
200
100
0
μ mhos/cm
Conductivity
9·0
8·0
7·0
pH
J F M A M J J A S O N D

Fig. 1.1.5

Myxophyceae and Euglenoidae; fungi like Aspergilus, Peziza, Morticrella and few unidentified ascomycetis and some aquatic weeds. The land area had predominant herbs belonging to species Polygonum hydropiper, Cichlorna cracepus, Hypochaeris radicata, Ambrosia artimesifolies, Eupatorium adenophoum, Canna indica and Artemisia species.

Being almost a natural habitat, a number of aquatic and terrestrial invertebrates and some vertebrates including pets and domesticated animals inhabit the area.

An analysis (technique described in chapter III) of the limnological conditions, such as pH, conductivity, dissolved bicarbonate, chloride, calcium, magnesium and total hardness of the pond water has been illustrated in Fig. 1.1.5. Correlation among the above mentioned limnological conditions have also been derived and presented in Table 1.1.2.

1.2 Pologround, adjoining Umkhrah river, Shillong (MEGHALAYA)
(1428 m.a.s.l. 25.34^{o}N and 91.56^{o}E)(Fig.1.2.1)

The Pologround site at Shillong has lotic water system, surrounded by hills having average slope of 30^{o} to 40^{o} and is situated about 1½ km north from State Transport Bus stop and south of Umpling colony. The

Fig. 1.2.1 : Map of the study site No. II - Pologround adjoining Umkhrah River, Shillong, Meghalaya.

SCALE
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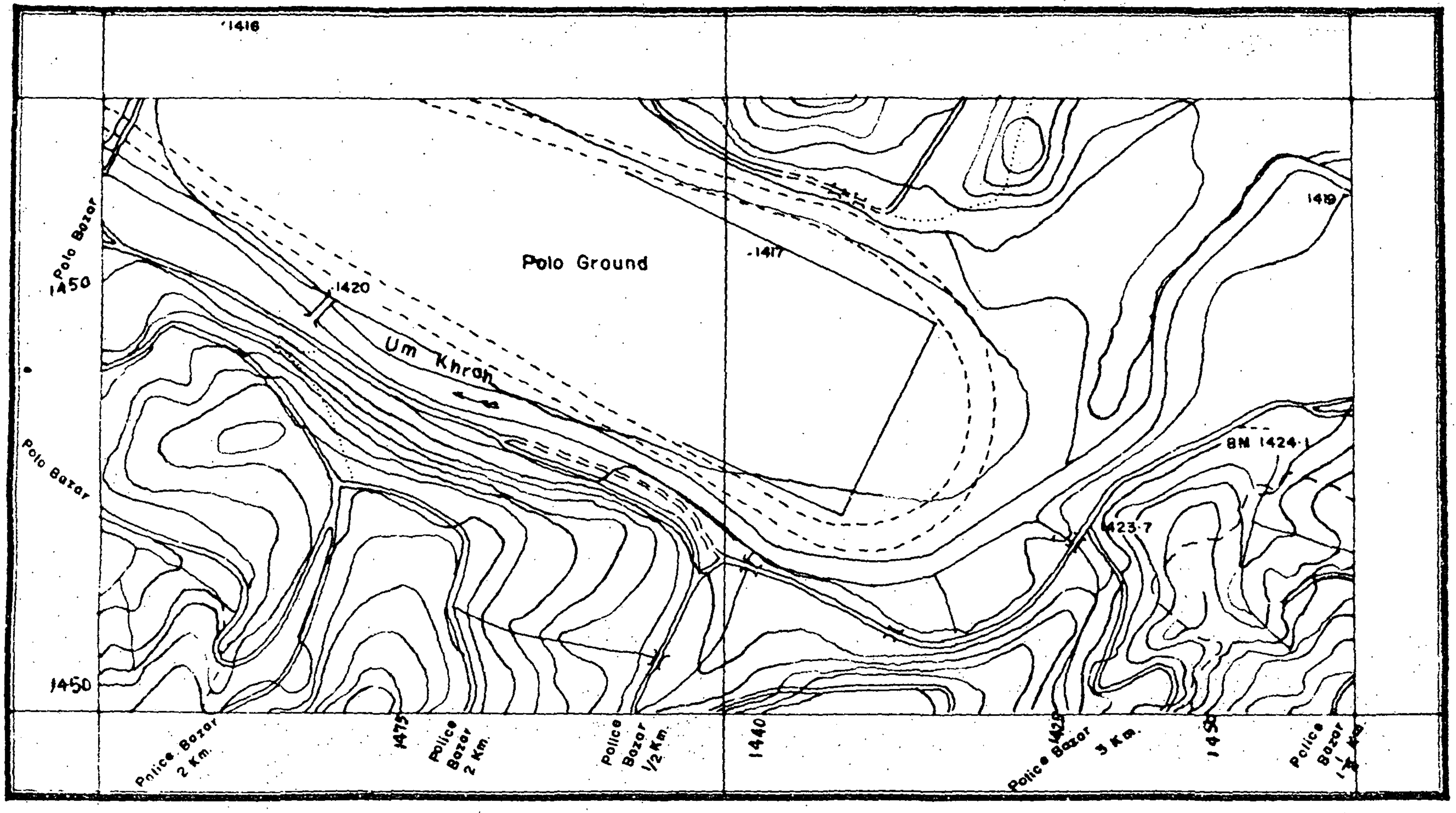

Fig. 1.2.1

Fig. 1.2.2 (A) & (B) : A view of the pond water of the study site No. II - Pologround adjoining Umkhrah river, Shillong, Meghalaya.

Fig. 1.22 A

Fig. 1.22 B

Umkharah river flows through the pologround area from south-east to north-west. It is 4.0 to 6.0 m wide and 0.5 to 1.0 m deep, from late June to late August, during rainy season the river overflows and floods the surrounding areas. The river has muddy and foul water due to excessive silting and sewage drained from the city (Fig. 1.2.2). The habitation and construction are very few, restricted to some hutment. The Pologround is used mostly for paddy and potato cultivation.

Shillong situated at the north of cancer has sub-tropical climate. Its Atmospheric and water temperature, humidity and rainfall are shown in Fig. 1.2.3 for the year 1978. The temperature varied between 1.3°C - 28.2°C. The average maximum atmospheric temperature was recorded to be 25°C in August and average minimum 4.5°C during January. The average water temperature at the site fluctuated between 6.7°C and 20.0°C and had minimum and maximum record of 4.0°C and 22.5°C in January and August respectively. The humidity at the study site fluctuated between 37% and 90% and was maximum during rainy season. Total rainfall during the year 1978 was recorded to be 1633.0 mm with a maximum precipitation of 460.1 mm during July. The winter months were dry with no rains. Correlations between different climatic factors have been shown in Table 1.1.1.

Fig. 1.2.3 : Environmental conditions at the Study Site No. II during 1978.

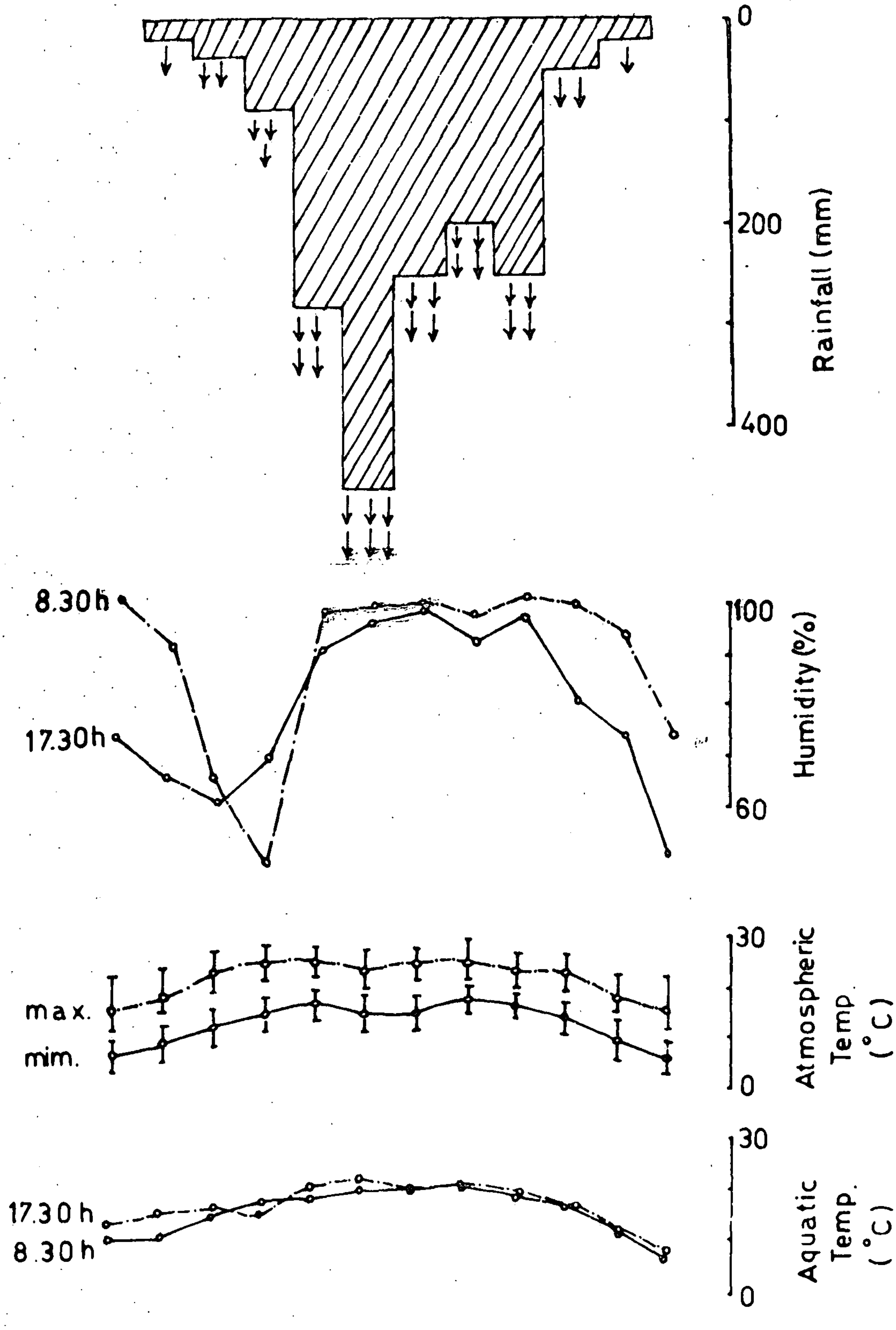
0
200
400
Rainfall (mm)
8.30h
17.30h
100
60
Humidity (%)
max.
mim.
30
0
Atmospheric Temp. (°C)
17.30 h
8.30 h
30
0
Aquatic Temp. (°C)

Fig. 1.2.3

Fig. 1.2.4 : Limnological conditions at the Study Site No. II during 1978.

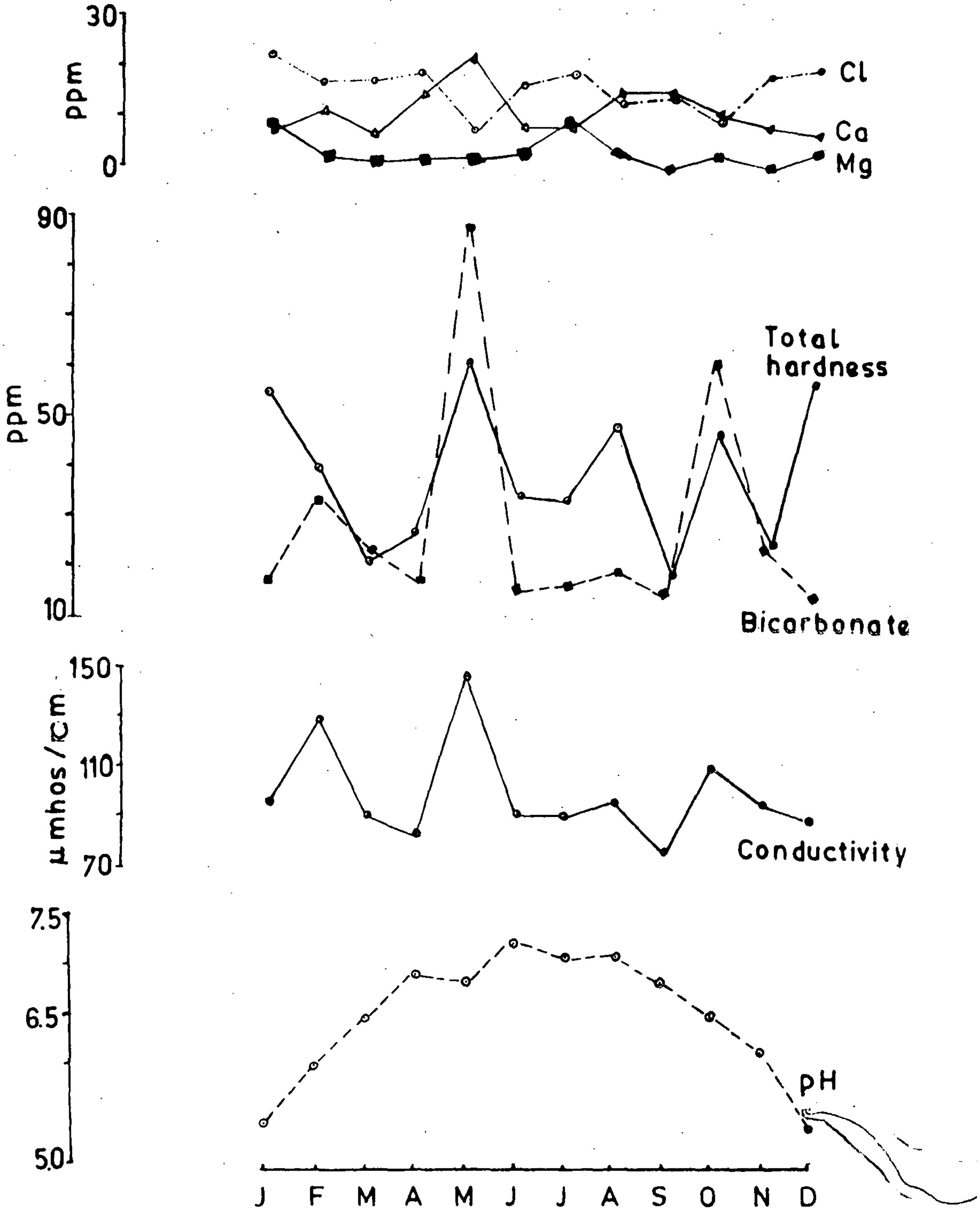
30
ppm
0
Cl
Ca
Mg
90
ppm
50
10
Total hardness
Bicarbonate
150
µ mhos / cm
110
70
Conductivity
7.5
6.5
5.0
pH
J F M A M J J A S O N D

Fig. 1.2.4

The vegetation at the site is broadly divided in four layers, trees, shrubs, herbs and ground layer. The trees and shrubs are very few belong to mainly Pinus and Hypericum species. The herbs that predominate the sites are Subrosia artimissifolia, Eupatorium adonopharum and Hypochaeris radicata, Artimisria species of the family Asteraceae; Polygonum hydropiper, Fagopyrum esculentum, Rumea nepalenses of family Polygonaceae; Alternanthera sessilis of Amaranthaceae; Drymaria cordata of Caryophylaceae; Galingsoga ciliata of Asteraceae; Canna indica of Cannaceae; Lantanna camara of Verbenaceae; Paspalum dilatalum of Poaecae and few Pouzolzia spp. of Urticaceae. At ground level the Aspergillus, Fusarium, Merticrella and few other ascomycites are recorded at its bankment.

Being almost a natural habitat a number of aquatic and terrestrial invertebrates and some vertebrates including pets and domesticated animals inhabit the area.

An analysis (technique described in Chapter 3[illegible]) of the limnological condition such as pH, conductivity, dissolved bicarbonate, chloride, calcium, magnesium and total hardness of the pond water has been illustrated in Fig. 1.2.4. Correlation among the above mentioned limnological conditions have also been derived and presented in Table 1.1.2.

Table 1.1.1

Correlation between various climatic conditions at the two study sites

GAUHATI					SHILLONG			
Rainfall mm	Humidity %	Aquatic Temp. °C	Atmospheric Temp. °C	Variables	Atmospheric Temp. °C	Aquatic Temp. °C	Humidity %	Rainfall mm
0.6541*	0.0817	0.85111**	-	Atmospheric Temperature °C	-	0.9217**	0.2704	0.6062*
0.8441**	0.3149	-	0.8511**	Aquatic Temperature °C	0.9217**	-	0.4990	0.6143*
0.4077	-	0.3149	0.0817	Humidity %	0.2704	0.4990	-	0.6247*
-	0.4077	0.8441**	0.6541*	Rainfall mm	0.6062*	0.6143*	0.6247*	-

* = Significant at 5% probability ($P < 0.05$)

** = Significant at 1% probability ($P < 0.01$)

<u>Table 1.1.2</u>

Correlation between various physico-chemical factors at the two study sites

GAUHATI						SHILLONG				
Total hardness ppm	Chloride ppm	Bicarbonate ppm	E.C. micromhos/mm	pH	Variables	pH	E.C. micromhos/mm	Bicarbonate ppm	Chloride ppm	Total hardness ppm
0.267	0.449	0.092	0.281	-	pH	-	0.054	0.076	0.454	0.391
0.963**	0.731*	0.844**	-	0.282	Electrical conductivity micromhos/mm	0.053	-	0.856**	0.575*	0.573*
0.911**	0.780**	-	0.844**	0.092	Bicarbonate ppm	0.076	0.856**	-	0.812**	0.492
0.754**	-	0.911**	0.731*	0.448	Chloride ppm	0.454	0.575*	0.812**	-	0.298
-	0.754**	0.911**	0.962**	0.268	Total hardness ppm	0.391	0.573*	0.492	0.298	-

* = Significant at 5% probability ($P < 0.05$)

** = Significant at 1% probability ($P < 0.01$)

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CHAPTER - 2

Diagnostic Characters, Morphometry and Annual Breeding Cycle

INTRODUCTION

Rana cyanophlyctis Schneider (Fig. 2) is closely related to *Rana hexadectyla* Lesson, though it is smaller in size and possesses perfectly webbed toes. The systematic position of *Rana cyanophlyctis* is as follows:

Phylum	-	Chordata
Sub-Phylum	-	Gnathostomata
Class	-	Amphibia
Order	-	Anura
Sub-order	-	Phaneroglossa
Series	-	Firmisternia
Sub-family	-	Ramidae
Genus	-	*Rana* Linnaeus
Species	-	*cyanophlyctis* Schneider

The first record of this species was made as early as 16th century when Emperor Babar, noticed that it skips over the surface of water like ricocheting stone (Daniel, 1975). Because of this peculiar habit it is popularly called "water skipper" or "skipper frog" and due to its aquatic habit it is also called "Pani Benga" or "water frog". It is a common and most easily available species inhabiting different biotopes throughout the Indian sub-continent. It is often seen floating on the surface

Fig. 2 : A female *Rana cyanophlyctis* Schneider.

2·0 cm

Fig. 2

or squatting along the edges of many ponds, rain puddlers, tanks, streams, temporary, perennial, running or stagnant clear or foul waterbodies and other such stretches. Annandale (1917) recorded them at Quetta (Pakistan) floating on a water body whose edges were frozen. McCann (1933) recorded them from cisterns and open gutters where the water was very foul, full of refuses, often discoloured with saffron curry material. He noticed them to move away from the drains when hot water was poured and returned when the water cooled. Daniel (1975) has reported them from water bodies having industrial effluents and brackish water.

Satyamurti (1967) has provided a detailed description of this species from South India and has mentioned it to be a widely distributed species from Malay Peninsula, up to Arabia including Nepal, India, Pakistan, Afganistan, Iran, Iraq, and Bangladesh, & in the north from Himalayas to Sri Lanka in south. Boulenger, Annandale Wall and Regan (1907) recorded it at an altitude of 4,500 to 6,400 feet above sea level (a.s.l.) at the hills from Bhimtal and Nainital. Acharji and Kriplani (1951) recorded it at Kangra and Kulu valleys in Darjeeling District and other parts of Eastern Himalayans at 6,000 feet a.s.l. Annandale (1909) recorded it at Kotagin and Nilgiri hills from 5,700 feet a.s.l. It is a common species of frog available in North-Eastern hills of India, Assam, Arunachal Pradesh and Manipur (Pillai and Chanda, 1976). In the present investigation, the frog has been recorded in abundance at Gauhati as well as at Shillong throughout the year.

Although detailed contributions are available on the biology, systematics and distribution of the *Rana cyanophlyctis* (Boulenger, 1890 and 1920; Annandale, 1909 and 1917; Narayan Rao, 1915 and 1920; McCann, 1933 and 1940; Satyamurti, 1967; Daniel, 1973 and 1975), information on morphometric analysis of the populations available at Gauhati and Shillong are lacking. More and more information has been pouring in on morphometric analysis of different anuran species (see Brown and Boschung, 1954, Duellman and Klass, 1964; Metter, 1964; Zweifel, 1964 and 1972; Hyers and Peter, 1971; Tyler and Martin, 1975 and Tinsley(1973 and 1975). Clarke (1974) and Mohanti-Hejmadi (1974) derived correlation between SV length and tibia length in *Rana tigrina* and *Bufo woodhousie*, Schroeder's (1975) contribution on the relationship between SV length and tympanum diameter at different age groups of *Rana catesbiana*, Terentjev's (1960) observation on relationship between SV length and fecundity, Koskela and Pasanen's (1975) contribution on regression relationship of different body parts of *Rana temporaria*, are some ofthe noteworthy contributions highlighting the significance of such investigations and intra-specific variations.

In the present chapter, an analysis of absolute measurements and morphometric ratios of various morphological structures of the samples of Rana cyanophlyctis populations collected at Gauhati and Shillong has been presented. This includes the description of morphological features and relationships between snout-vent length (SVL) and body weight (BW) of the two samples of this species. A 12-month analysis of its relative condition has been worked out to know the size availability and robustness of the frogs during different months of the year. In this connection, coefficient correlation of the two variables mentioned has been been worked out. In addition to these an account of its annual breeding cycle has also been included.

REVIEW OF LITERATURE

Boulenger (1882) classified and catalogued the Batrachia, Sailentia and Eucaudata collected at the British Museum on the basis of their morphological characters. The first contribution on the morphology of the Indian anurans seems to be in the year 1888 and that of Dr. Edgar Thurston the then superinetendent, Government Central Museum Madras. He compiled a concise book entitled "Catalogue of the Batrachia, Sailentia and Apoda of Southern India". It contained descriptions of thirteen plates of the specimens present in the collections of Madras Museum and British Museum of Natural History, London. This book is now out of print and also out of stock (Satyamurti, 1967). Boulenger's monumental monograph "Reptiles and Amphibia" published in 1890 in the Fauna of British India is an record on the anurans of this continent. It is even today considered as a standard reference for the study of anuran morphology, taxonomy and systematics. This monograph is also out of print and its copies available in the libraries and museums are often so fragile that one cannot use them freely for reference.

Boulenger (1882, 1890 and 1920), Thurston (1888), Annandale (1909 and 1917), Narayan Rao (1915 and 1920), McCann (1933, 1940 and 1945), Acharji and Kripalani (1951) and other earlier workers contributed mainly to the morphology, taxonomy, distribution, habits and behaviour of the anurans. The morphology was often restricted to provide the diagnostic characteristics explained with the help of absolute measurements. Workers like Bragg(1950) and Taylor (1951) added absolute measurements of many more body parts for the study of morphology apart from analysing their diagnostic characters in both sexes. Later, Brown and Boschung (1954), Duellman and Klass (1964), Metter (1964), Zweifel (1964), Tinsley (1973, 1975) used absolute measurements as well as ratio study for the morphology. Morphologist like Heyer and Peters (1971), Zweifel (1972), and Tyler and Martin(1975) used absolute measurements and ratio analysis for the study of races in population, collected from different ecological conditions and lastly workers like Clarke (1974) and Tahin *et al.* (1977) used correlation between two different structures. Pasanen and Koskela (1974) and Koskela and Pasanen (1975) investigated relationship between SV length and various biometrics of *Rana temporaria* and thereafter provided regression equations enabling to calculate the weight of ovaries, weight of oviduct, egg number and egg size, from the known measurements of SV length and body weight of the frog.

The important review of literature for the past two decades are as follows:

Bragg (1950) in *Bufo congnatus*, Tayler (1951) in *Bufo simus* and Brown and Boschung (1954) in *Rana palustris*, made morphological study and absolute morphological measurements to describe the morphological variations in populations inhabiting at different environmental conditions. Brown and Alcala (1963) applied the following three ratios (1) breadth of third finger disc/length of third finger; (2) Head width/Snout-Vent length; and Head width/length of Tibia along with absolute morphological variations and description for the identifications of a new frog belonging to genus Cornufer (Ranidae). On the morphological analysis of the skin structure colourations, 7 absolute measurements, and 6 morphological ratios in hylid frog populations Duellman and Klass (1964) recorded a new species Triprion petastus. Describing the utility of the ratio study in animals Cochran (1953) remarked that morphological ratios studies provide a better understanding of the morphological structure and variations, it also compensates the natural variation of sizes. He noted that an average ratio, derived, by finding the specific ratio for each individuals and then dividing the total by the number of individuals was less accurate than the ratio obtained by dividing the sum of numerators by the sum of denominators. Metter (1964) applied Cochran's technique to 8 ratios and 10 absolute measurements for a morphological comparison of the two populations of tailed frog Ascaphus truci. Similarly, Zweifel (1964) in Rana viticaria and Martin and Littlejohn (1966) in Hyla jervisiensis used ratio study to describe the morphology of the frog. Heyer and Peters (1971) observed the synonyms in Leptodactylids collected from Ecuador, and Zweifel (1972) reviewing the available preserved specimens of genus Lechrides used various indices of ratio and absolute measurements, for morphological understanding. Similarly the population studies in Bufo mexicanus (Webb, 1972) and Leptodactylids (Tyler and Martin, 1975) were made by analysing the morphological ratios and absolute counts. Tinsley (1973 and 1975) used 9 indices, showing ratios between various body part and 18 absolute measurements for the study of the biology, systematic and synonyms in Xenopus laevis, Xenopus vestitus, Xenopus bunyoniensis, Xenopus victorianus and Xenopus kigesiensis . However, Van Dijk (1966) showed the utility of the ratio in the systematic studies of anuran larvae.

Some references on the morphology and measurements of Indian anurans of the recent decades can be reviewed as follows:

Daniel (1963a, 1963b, 1975) described habit and habitat, distribution and morphology of anurans from western India, Satyamurti (1967) from southern India, Behura (1965) and Mohanty-Hejmadi (1974) from Orissa. Pillai and Chanda (1973, 1976) described 40 amphibian species from North-eastern India. The description contained 3 anuran records namely Rana danieli, Rana mowplangensis and Philautus shillongensis identified with the help of 22 absolute and 6 ratio morphological counts. Dubois (1976) collected anuran fauna from Nepal and recorded the presence of two uncommon black eye *Rana cyanophlyctis*. Recently Roy (1979) made morphological description of *Rana limnocharis* of Shillong population with the help of absolute and ratio morphological measurements as described by Tinsley (1973, 1975).

These days morphological characters are frequently accompanied by biometric studies. Among anurans such studies are very few specially when compared to fishes. Rao (1964) while describing *Hilsa kanagenta* applied (1) length frequency distribution i.e. the occurrence of more or less same length group in different season. It also reflects the size group of animals predominating the habitat during different months, (2) length weight relationship, i.e. length group (cm) plotted against the corresponding average weight (gm). In the above two bivariates (length and weight) showed linear relationship and has been expressed by $W = a + bL^3$ (where W and L being weight (gm) and length (cm) of the animal respectively and a and b as equation constants). Similar length frequency distribution and length weight relationship have also been studied in Gizzart Shad (Babu Rao, 1965) in *Otolithoides microdon* (Sinha and Rao, 1965); in *Stipinna godavariensis* (Rao, 1967) and in *Hilsa ilisha* (Rao, 1969).

LeCren (1951) derived a relationship between body length and weight $K = \frac{W}{L^3} \times 10^5$ and noted that the relative conditions of the organism can be predicted with the help of the above equation. Following the above technique, the relation condition has been estimated in various animal such as <u>Hilsa kangenta</u> (Rao, 1964); in <u>Otolithoides microdon</u> (Sinha and Rao, 1965) and in <u>Hilsa ilisha</u> (Rao, 1969). It is observed that in all the above cases the maximum relation condition (constant) has been observed during spawning period.

Bayless (1969) plotted regression equations and relationship between SV length and Tibia length of <u>Ascris crepitans</u> and compared it with <u>Bufo woodhousei floweri</u> . Tinsley (1973) derived various biometric relationship in body part of <u>Xenopus kigesiensis</u> Schrocder (1974) in the body part and Tympanum length of adult <u>Rana catesbeiana</u> . Clarke (1974) used Tibia measurements as a growth indicative in <u>Bufos</u> and noted that SV length and Tibia length have significant correlation, with high correlation coefficient of ($r = 0.998$) . Labanick and Schlucler (1976) observed that relationship between tibia length and SV length appeared to be linear and can be expressed by least square regression lines $y = 2.37x + 1.45$, when y and x are Tibia and SV length respectively. Koskela and Viro (1976) observed linear relationship in body length with animal weight and with tail length in harvest mouse.

A method for racial analysis suggested by Mahalonobis (1936) has been followed by certain workers. He mentioned that the distance/difference between the two population, D_p^2 can be estimated by the variance-covariance analysis and comparison of the variables and means difference of the two populations. Kesteven (1950) noticed that D_p^2 analysis on statistical 'F' test, if yields values, below or equal to 1%. Probability level (P = 0.01), indicates that the populations inhabiting in two environments are morphometrically non distinct and indiscriminent functions can be developed between them. Such population can be considered as homogeneous stock. Rao (1952) and Keeping (1964) further reported that covariance and mean difference in the variables of the two populations can be used for the racial studies. Gupta (1970) applied the D_p^2 analysis (Mahalonobis, 1936; Rao, 1952) in the meristic counts of different populations of Polynernus paraliscus for the racial study. Similarly, Pathak (1979) took help of the above described technique for the reacial study in Cirshinus riba collected from two different hydrographical and physico-chemical conditions, namely Ganga and Yamuna riverines and noted minor structural difference in the two populations of the fish, although not statistically significant on D_p^2 analysis, to assign them two different races.

MATERIAL AND METHODS

Adult specimens of Rana cyanophlyctis were collected at the study sites at Gauhati and Shillong at regular intervals throughout the year. After collection the Snout-Vent length (SVL) of each frog was recorded and its body weight (BW) was taken after blotting the body surface. Their colour pattern was noted and they were killed with chloroform, their guts and gonads were removed and preserved in 10% formalin.

Range, mean, standard deviations of various measurements were calculated separately for samples of two populations in various size groups of male and female to compensate for the natural variations in sizes.

(a) Absolute measurements of the morphological character:

For females, following 17 and for males following 18 absolute measurements were recorded with the help of divider to the order of 0.1 mm accuracy.

(1) Snout-Vent Length(SVL) : The measurement between tips of snout and vent of the animal.

(2) Body width (B.W.)	:	Measurement of the widest part of the body.
(3) Head length(H.L.)	:	Measurement from the anterior length of the animal to occipital condyl.
(4) Head width (H.W.)	:	Measurement at the widest region of the head.
(5) Snout length (S.L.)	:	Measurement of the perpendicular distance from the tip of the nose to the anterior most end i.e. the anterior level of the premaxillae bone.
(6) Snout width (S.W.)	:	The measurement of the widest point of the snout lying just below the nose.
(7) Eye diameter(E.D.)	:	The transverse distance across the exposed eye orbit.
(8) Inter ocular distance (I.O.D.)	:	The shortest distance between two eyes.
(9) Tympanum diameter (T.D.)	:	The measurement between annulus tympanicus across tympanic membrane.
(10) Inter tympanal distance (I.T.D.)	:	The shortest distance across head between two tympanic membranes.
(11) Inter narial distance (I.N.D.)	:	The distance between the inner margins of the flabs bordering nostrils.

(12)Hind limb length (H.L.L.)	:	Total distance from the vent to the tip of the 4th toe.
(13)Tibia length (T.L.)	:	Median measurement along the dorsal surface of tibia.
(14)4th toe length (4.t.l.)	:	Measurement of the outer ventral surface of the 4th digit.
(15)Forelimb length (F.L.L.)	:	Measurement from the origin of forelimb to the tip of 1st finger.
(16)Radio-Ulna length (RU.L.)	:	Median measurement along the dorsal surface of the radio-ulna.
(17)1st finger length (F.L.)	:	Measurement from the base to the tip of the 1st digit.
(18)Vocal slit's length (V.St.L.)(only for males)	:	Measurement from the anterior edge to the posterior edge of the slit.

(b) Morphometric ratios:

Cochran's (1953) method has been applied for the ratio count analysis of the frog. The ratio has been obtained for each count by dividing the sum of the total numerator by the sum of total denominator. Following the ratio counts were estimated:

(1) SV length/Hind limb length (SVL/HLL)
(2) SV length/Snout length (SVL/SL)
(3) SV length/Snout width (SVL/SW)
(4) SV length/Tympanum diameter (SVL/TD)
(5) SV length/Head width (SVL/HW)
(6) SV length/Eye diameter (SVL/ED)
(7) Head length/Head width (HL/HW)
(8) Tibia length/Hind limb length (TL/HLL)
(9) 4th toe length/Total fore limb length (4th L/FLL)
(10) Lower fore limb length/Total fore limb length(LF/FLL)
(11) Head length/Internarial distance (HL/IND)
(12) SV length/Total fore limb length (SVL/FLL)
(13) Snout width/Snout length (SW/SL)
(14) Internarial distance/Inter tympanum distance (IND/ITD)
(15) Head width/Inter tympanum distance (HW/ITD)

(c) <u>Length-weight relationship:</u>

For length and weight relationship, graphs were plotted with length on 'X' axis and weight on 'Y' axis. From the trend of the distribution of the observations, the equation with the minimum sum of the squared differences between length and weights were taken as the best fit, regression expression. The correlations (r-value) between length and weight were calculated for males and females separately as well as together. The length and weight measurements of both the sexes, were applied to LeCren's (1951) formula given below and subsequently the equation constant, namely initial growth index (c) and equilibrium constant (n) were found out.

$$W = cL^n$$

$$\log W = \log c + n \log L$$

where

W = weight

L = length

c = initial growth constant

n = equilibrium constant

(d) Relative conditions:

The relative condition variations in the mixed samples of frog population, collected at Shillong and Gauhati from January to December, was calculated, with the help of LeCren's (1951) formula

$$K = \frac{W \times 10^5}{L^3}$$

where

K = condition factor

W = weight of frog

L = length of frog

The condition factor so derived was also correlated with environmental conditions of the sites such as temperature, relation humidity and rainfall, separately and jointly.

...... Contd P. 34/-

OBSERVATIONS

The observations include:

Diagnostic Features and Colour pattern, Distinguishing characters of male and female *Rana cyanophlyctis*, Morphometric measurements, (Absolute measurements and Morphometric ratios), of Gauhati and Shillong population of *Rana cyanophlyctis*, Length-Weight relationship and Relative condition and Annual breeding cycle.

2.5.1 Diagnostic Features:

Rana cyanophlyctis is characterised by following features.

1. Snout blunt, more or less rounded, scarcely projecting beyond mouth (Figs. 2.1, 2.2, 2.9 & 2.12).
2. Head flattened and compressed dorsoventrally (Fig. 2.12).
3. Head, slightly broader than long in adults and as long as broad among immature and juveniles (Figs. 2.1, 2.2, 2.10 & 2.12).

Fig. 2.1 : Dorsal view of Rana cyanophlyctis - female
SNT = Snout; TYM = Tympanum; WTS = Warts;
F.L = Fore limb; H.L = Hind limb.

2.2 : Dorsal view of Rana cyanophlyctis - male
Na = Narial; VS = Vocal sac.

2.3 : Ventral view of Rana cyanophlyctis - female
Ab = Abdomen; Wb = Web.

2.4 : Ventral view of Rana cyanophlyctis - male
VS = Vocal sac; Ab = Abdomen.

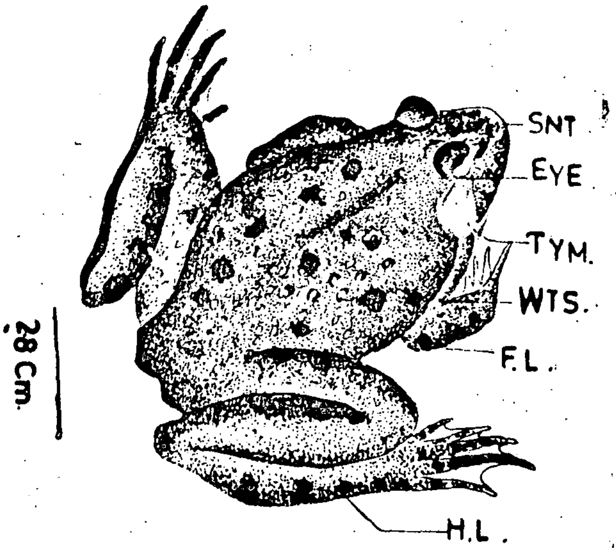

Fig. 2.1

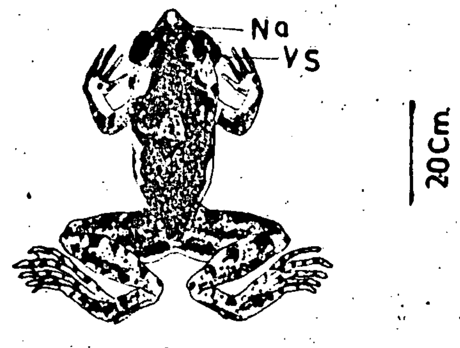

Fig. 22

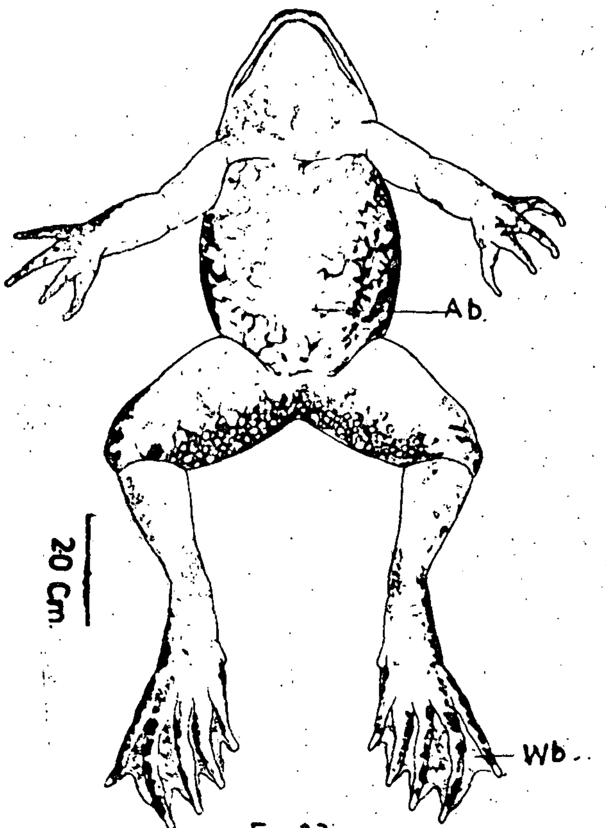

Fig. 23

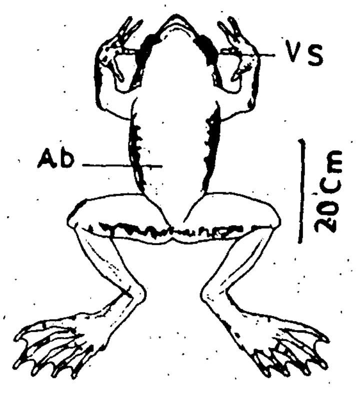

Fig. 24

4. Nostrils at equal distance from eyes and from the tips of the snout (Figs. 2.2 & 2.12).

5. Distance between nostrils approximately equal to the inner orbital width, however, narrower than the distance between the upper eyelids (Figs. 2,2 & 2.12).

6. Tympanum distinct and approximately 3/4 of the total eyelid diameter (Figs. 2.2, 2.10 & 2.12).

7. Inner orbital space much smaller than eyelids.

8. The vomerine teeth are disposed in small round or oblique groups and situated at level with posterior border of the choanae, or just behind them.

9. Skin warty with small tubercles and with rows of pores dorsally, smooth and colourless ventrally (Figs. 2.1, 2.4, 2.12, 2.9 & 2.13).

10. Flanks warty with pores arranged in single line (Figs. 2.3, 2.4 & 2.12).

11. Presence of a strong skinfold between the eye and the shoulder (Figs. 2.1 & 2.2).

12. Subarticular tubercles small and feebly prominent. The tarsal folds either absent or poorly developed with small dermal fringes present on outer toe (Figs. 2.5 & 2.14).

13. Outer tubercles absent but pointed digitiform inner metatarsal tubercles present (Figs. 2.3 & 2.5).

Fig. 2.5 : Hind limb of _Rana_ _cyanophlyctis_ - female

Wb = Web.

2.6 : Fore limb of _Rana_ _cyanophlyctis_ - male

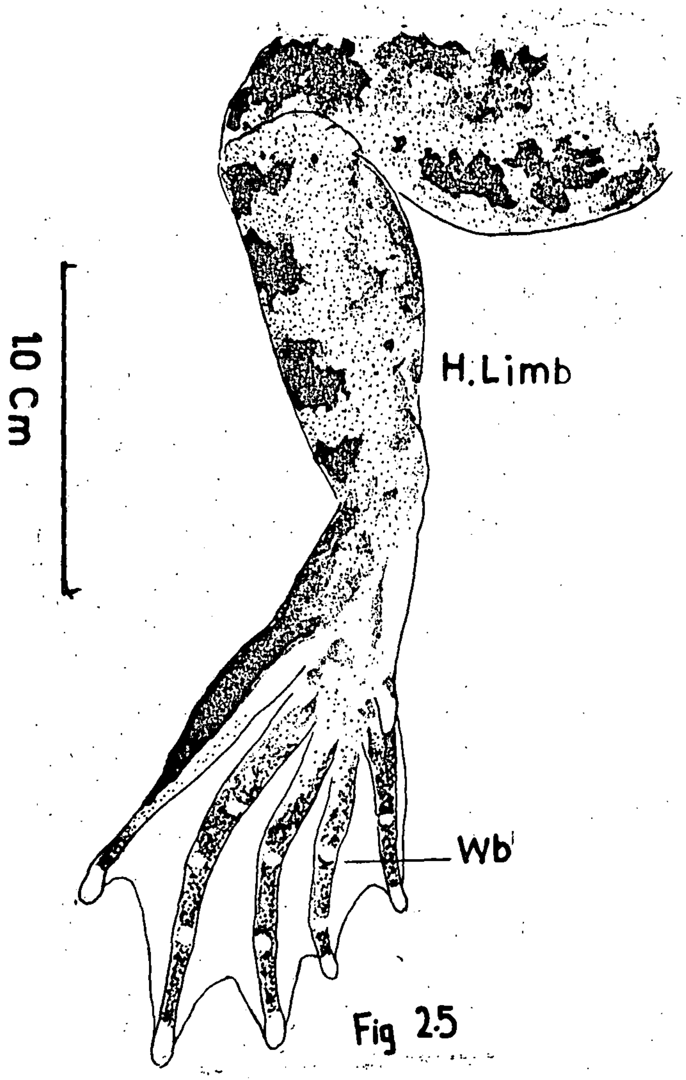

Fig 25

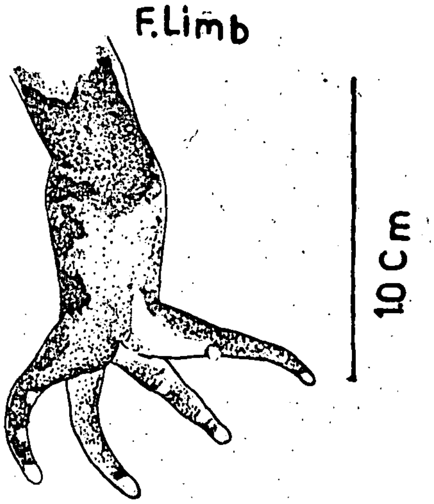

Fig 26

14. Fingers thin pointed and feebly developed (Figs. 2.6 & 2.13).

15. First and second fingers almost equal in length (Figs. 2.6 & 2.13).

16. Toes completely webbed (Figs. 2.5 & 2.14).

17. Toe tips swollen, rounded or dilated into very small discs having well developed broad web reaching the tips (Figs. 2.5 & 2.14).

18. The 4th toe moderately large than others (Figs. 2.5 & 2.14).

19. Inner metatarsal tubercles, small elongated and conical (Figs. 2.5 & 2.14).

20. Outer metatarsals separate up to their bases (Fig. 2.14).

2.4.1 (b) Colouration:

Normally the codour pattern of the animal seems to be dependent on its surroundings. The riparian collected from foul and dirty place have dull colouration as compared to those collected from clean running water. The colouration of head and body on the dorsal side varies from brownish to dirty green, greyish or olive-brown, spotted or marbled with numerous dark olive marking of irregular shape (Figs. 2.1, 2.2, 2.8 & 2.12).

Fig. 2.13 : Fore limb of *Rana cyanophlyctis* - male. × 1.0

2.14 : Hind limb of *Rana cyanophlyctis* - male × 1.0

Fig. 2.13

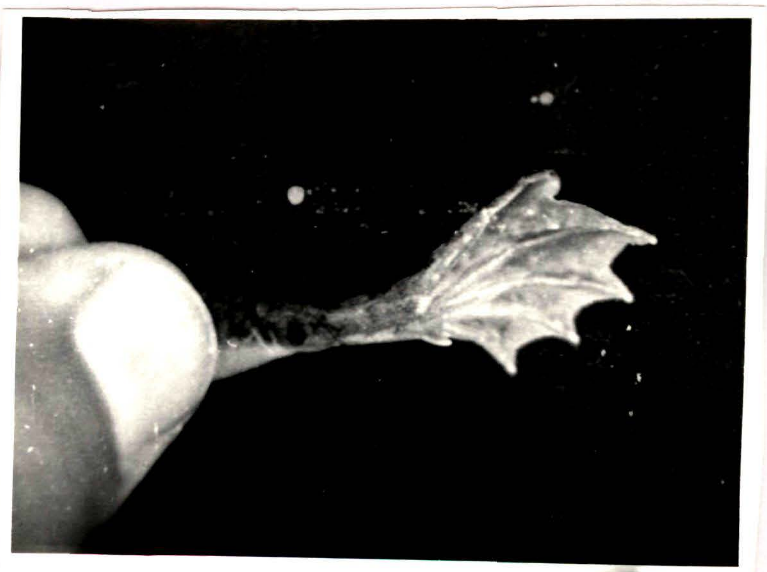

Fig. 2.14

On the head, however, the number of black markings are less. Colouration of limbs is like rest of the body. Ventral aspect of the limbs is creamy white or creamy tans (Figs. 2.4 & 2.9). Incomplete dark brown cross bands are present on the limbs (Figs. 2.1, 2.2, 2.12& 2.10). The upper part of fore limbs are often lighter in colour than their lower parts. Flanks have identical colour like that of dorsum although bit lighter in shade. Often one or two black streaks with white margin are present at the hinder aspect of the thighs below the vent (Figs. 2.4 & 2.11). There are feebly distinct light black edges and bands on each flanks and thighs, which being more prominent in males and juveniles than females. Chin, throat, belly (Fig. 2.13) and other ventral surfaces are creamy white, pale yellowish or dirty white (Figs. 2.4 & 2.9). The ventral surface of the juveniles are without markings or spots . The large size adults often have marbled spotted, dotted or vermiculated surface with black colouration on the dorsolateral region (Figs. 2.3 & 2.11). Such dots are sometimes seen on the ventral surface of the throat and belly also (Figs. 2.3 & 2.11).

2.4.2 Distinguishing characteristics of the males and females:

Following are distinguishing characteristics of

Fig. 2.7 : A comparison of male and female adult *Rana cyanophlyctis* × 1.0

2.8 : Lateral view of *Rana cyanophlyctis* - male. × 2.0

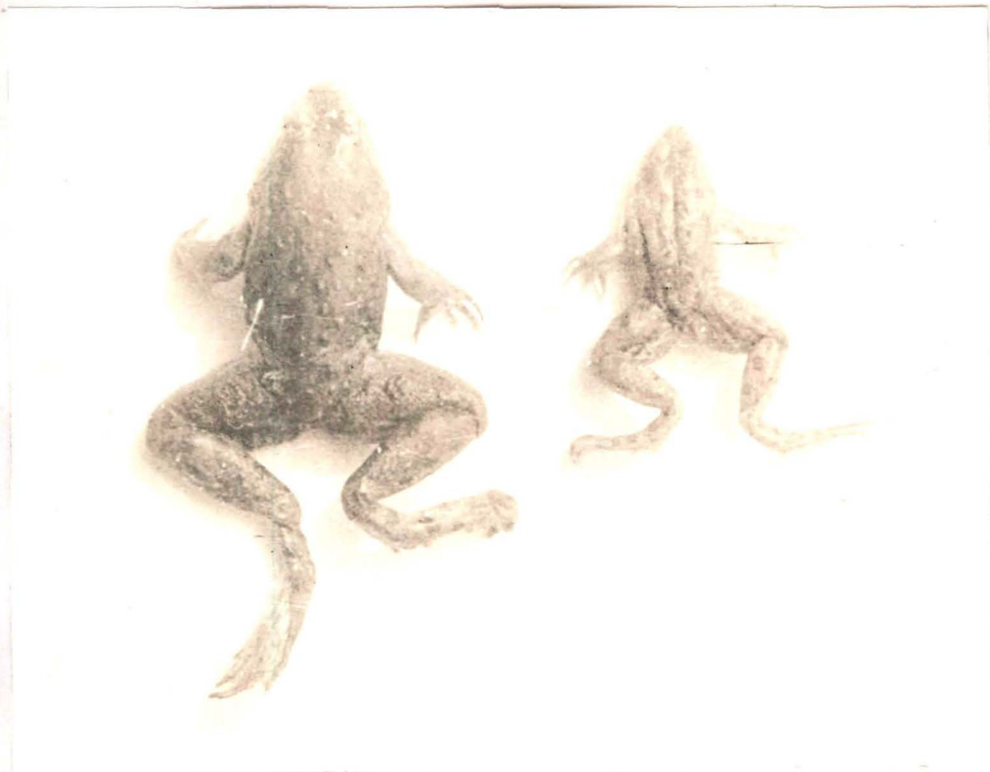

Fig. 2.7

Fig. 2.8

males and females.

(a) Males (Figs. 2.2, 2.4, 2.9 & 2.10)

1. The males are identified by their small size (SVL from 4.0 cm to 5.20 cm) and weight from 7.0 gm to 19.0 gm (Fig. 2.7).

2. They possess prominent greyish or blackish external vocal sacs on each side of throat, close to the posterior half of the mandibular ramus. The sacs are enclosed in separate slits and project out while croaking. The size of slit approximately equal to the 1st finger and larger than eye ball.

3. They produce "breeding call", apart from occasional "pain release call". The breeding call resumbles, some what low pitched rattles of bones kept up for a short while (McCann, 1933)

4. The 1st finger is slightly enlarged and thickened than others. Thumb pads, as seen in other frog species during breeding seasons are not present in Rana cyanophlyctis.

5. The colouration of males is brighter than that of female in the same population.

6. Their black and dark oliver marking on back are more conspicuous than those on females.

Fig. 2.10 : Dorsal view of *Rana cyanophlyctis* - male × 1·5

2.9 : Ventral view of *Rana cyanophlyctis* - male × 1·5

Fig. 2.10

Fig. 2.9

7. The black streaks with white granular bodies found in females are either absent or feebly developed in males.

8. Their abdomen is slender, lesser in diameter than pectoral region.

9. While sitting they keep their head above the body line.

10. They are active swimmers and are often seen floating on water surface.

11. They and their juveniles occupy the territorial edges of the deep water bodies.

12. They are active during day as well as night almost throughout year.

(b) Females (Figs. 2.1, 2.3 & 2.12)

1. They are much larger and heavier than males. SVL ranging from 5.10 cm to 7.20 cm and weight from 22.0 gm to 38.5 gm (Fig. 2.7).

2. They are not capable of producing any voice except rare "pain or release call".

3. Their fingers do not show any difference at any period.

4. Their skin possesses larger number of tubercles, warts and pores than that of males.

Fig. 2.11 : Ventral view of <u>Rana</u> <u>cyanophlyctis</u> - female x 0.66

2.12 : Dorsal view of <u>Rana</u> <u>cyanophlyctis</u> - female x 0.66

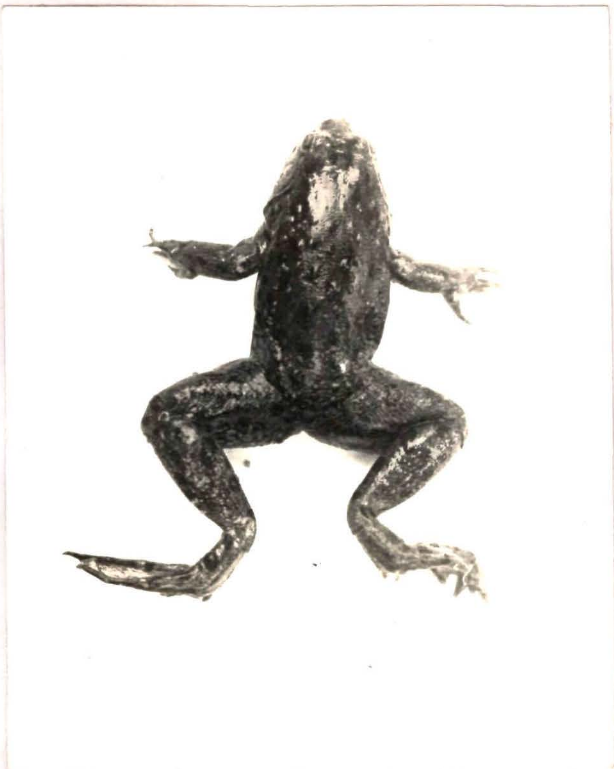

Fig. 2.12

Fig. 2.11

5. They are dull coloured when compared with that of males in the same population.

6. The black dark olive marking on their back are either feebly developed or absent.

7. Their black streak with numerous white granular bodies on the vent is more conspicuous and better developed than in males.

8. Their abdomen is often swollen and larger in diameter than pectoral region.

9. They appear more shy and sit with body and head approximately parallel to the substrate.

10. They are less agile and during leisure are found sitting on the edges of the water bodies. On approach they skip and dive in water bodies for hiding.

11. They prefer middle core of the deep water bodies.

12. They do not feed during breeding phase and spawning.

[illegible] <u>Morphometric measurements:</u>

The morphometric measurements were taken in samples of mixed specimens collected all around the year.

Table 2.1

Body dimensions of <u>Rana cyanophlyctis</u>

(Shillong population)

S.No.	Characters	Males (sample size 35)			Female (sample size 43)		
		Range (cm)	Mean (cm)	S.D.	Range (cm)	Mean (cm)	S.D.
1.	Body length (SV length)	4.00 - 5.30	4.830	0.4985	5.15 - 7.15	6.337	0.6326
2.	Body width	1.45 - 2.35	1.860	0.2158	2.05 - 4.20	3.091	0.7383
3.	Head length	1.25 - 1.90	1.667	0.1608	1.70 - 2.50	2.273	0.1871
4.	Head width	1.05 - 1.61	1.315	0.1826	1.60 - 2.65	2.316	0.2647
5.	Snout length	0.25 - 0.40	0.331	0.0403	0.30 - 0.55	0.436	0.0515
6.	Snout width	0.40 - 0.70	0.624	0.0547	0.65 - 0.95	0.795	0.0705
7.	Eye diameter	0.30 - 0.60	0.494	0.1743	0.50 - 0.80	0.640	0.0619
8.	Inter orbital distance	0.20 - 0.25	0.222	0.0305	0.25 - 0.40	0.323	0.0413
9.	Tympanum diameter	0.30 - 0.45	0.380	0.0502	0.40 - 0.60	0.530	0.0513
10.	Inter tympanum diameter	1.00 - 1.35	1.181	0.0840	1.20 - 1.80	1.591	0.1508
11.	Inter narial distance	0.25 - 0.40	0.332	0.0382	0.35 - 0.50	0.408	0.0392
12.	Hind limb length	6.05 - 8.50	7.315	0.5852	0.85 -11.05	9.857	0.8219
13.	Tibia length	1.40 - 2.40	2.085	0.2832	2.00 - 3.50	2.968	0.3472
14.	4th toe length	2.00 - 2.50	2.267	0.0954	2.25 - 3.60	3.006	0.3157
15.	Total fore limb length	2.10 - 2.80	2.462	0.1554	2.30 - 3.60	3.225	0.3266
16.	Lower fore limb length	1.45 - 1.85	1.644	0.1129	1.50 - 2.55	2.195	0.2235
17.	1st finger length	0.70 - 1.00	0.834	0.0591	0.70 - 1.25	0.925	0.1411
18.	Slit length	0.65 - 1.05	0.877	0.1352			

(a) Absolute measurements:

(i) Shillong specimens

Measurements of 77 frogs (35 males and 43 females) were taken. The SV length of the males ranges from 4.0 to 5.2 cm and that of the females 5.10 cm to 7.20 cm. The measurements of 18 body parts for males and 17 for females are illustrated in Table 2.1.

(ii) Gauhati specimens

Measurements of 54 frogs (20 males and 34 females) were taken. The SV length of the male ranges from 4.0 cm to 5.0 cm and that of the females from 5.0 to 6.75 cm. The measurements of 18 body parts for males and 17 for females are illustrated in Table 2.2.

A comparison of the measurements of the two types of samples reveal that on an average, the samples of frogs examined, both males and females were larger in size at Shillong than at Gauhati. But the males showed some distinguishing features. The head of males collected at Shillong, particularly snout length was smaller than those of the Gauhati frog by about 0.01 cm. This is reflected by other features, such as eye diameter: inter narial distance and tympanum diameter. The average lower fore limb length was also less in the male frogs of Gauhati by about 0.04 cm .

Table 2.2

Body dimensions of <u>Rana cyanophlyctis</u>

(Gauhati population)

S.No.	Characters	Males (sample size 20)			Female (sample size 34)		
		Range (cm)	Mean (cm)	S.D.	Range (cm)	Mean (cm)	S.D.
1.	Body length (SV length)	4.00 - 5.10	4.530	0.3412	5.00 - 6.75	5.841	0.5285
2.	Body width	1.30 - 2.00	1.685	0.2306	1.70 - 3.30	2.541	0.4655
3.	Head length	1.30 - 2.00	1.592	0.2199	1.70 - 2.55	2.097	0.2097
4.	Head width	1.25 - 2.05	1.545	0.2350	1.70 - 2.70	2.107	0.2716
5.	Snout length	0.30 - 0.40	0.347	0.0379	0.30 - 0.45	0.401	0.0398
6.	Snout width	0.45 - 0.70	0.575	0.0697	0.60 - 0.80	0.702	0.0475
7.	Eye diameter	0.40 - 0.60	0.507	0.0437	0.50 - 0.70	0.572	0.0495
8.	Inter distance orbital	0.20 - 0.30	0.252	0.0302	0.25 - 0.35	0.263	0.0309
9.	Tympanum diameter	0.30 - 0.50	0.410	0.0640	0.40 - 0.60	0.495	0.0450
10.	Inter tympanum diameter	1.00 - 1.25	1.130	0.0784	1.20 - 1.70	1.463	0.1388
11.	Inter narial distance	0.30 - 0.40	0.327	0.0302	0.30 - 0.45	0.392	0.0304
12.	Hind limb length	7.15 - 7.60	7.215	0.3433	7.45 -10.65	9.233	1.0615
13.	Tibia length	1.95 - 2.35	2.077	0.2061	2.15 - 3.25	2.714	0.3794
14.	4th toe length	1.80 - 2.40	1.932	0.4053	2.30 - 3.20	2.755	0.3081
15.	Total fore limb length	2.40 - 2.80	2.400	0.1898	2.60- 3. 60	3.166	0.2886
16.	Lower fore limb length	1.35 - 2.00	1.680	0.1969	1.75 - 2.40	2.132	0.1804
17.	1st finger length	0.65 - 1.05	0.847	0.1105	1.00 - 1.25	1.123	0.0931
18.	Slit length	0.60 - 1.00	0.815	0.1148	1.00 - 1.25	1.123	0.0931

(b) Morphometric ratios:

The morphometric ratios of various body parts have been compiled in Table 2.3. The SV length of males frogs from Shillong population ranges 4.00 cm to 5.30 cm and those of Gauhati frogs ranged from 4.00 cm to 5.10 cm and female frogs from Shillong population showed SV length ranges from 5.15 cm to 7.15 cm and Gauhati frog population from 5.00 cm to 6.75 cm.

Morphometric ratios among Shillong frogs population were more than Gauhati. However, few ratios such as SVL/SW (7.87), LFL/FLL (0.70) and IND/ITD (0.29) of Gauhati males were higher than those observed in Shillong frog population. Such measurements being 7.74, 0.67 and 0.28 respectively. Female frogs of Gauhati had higher SVL/SL (14.55), SVL/SW (8.31), SVL/HW (2.77), SVL/ED (10.21), HL/HW (1.00), HL/IND (5.36) and IND/ITD (0.27) that those of Shillong females. These ratios in Shillong females were 14.53, 7.96, 2.73, 9.89, 0.98, 5.16 and 0.26 respectively.

2.4.3 (a) Length weight relationship:

Length and weight relationship has been worked out for a sample of 30 male and 30 female frogs. The SV length of males varied from 4.05 cm to 4.90 cm and

Table 2.3

Morphometric ratio of <u>Rana cyanophlyctis</u> from two population (1) Shillong (2) Gauhati

S. No.	Character's for ratio SV length(Range)	Male Shillong (4.00 - 5.30)	Male Gauhati (4.00-5.00)	Female Shillong (5.15 - 7.15)	Female Gauhati (5.00-6.75)
1.	Snout-vent length/Hind limb length	0.6602	0.6278	0.6425	0.6326
2.	Snout-vent length/Snout length	14.5732	13.0359	14.5253	14.5494
3.	Snout-vent length/Snout width	7.7368	7.8782	7.9634	8.3096
4.	Snout-vent length/Tympanum diameter	12.7105	11.0487	11.9451	11.7863
5.	Snout-vent length/Head width	2.9893	2.9320	2.7344	2.7718
6.	Snout-vent length/Eye diameter	9.2377	8.9261	9.8856	10.2107
7.	Head length/Head width	1.0318	1.0307	0.9814	1.0006
8.	Tibia length/Hind limb length	0.2851	0.2879	0.3050	0.2984
9.	4th toe length/Fore limb length	0.3099	0.2678	0.3050	0.2984
10.	Lower fore limb length/Fore limb length	0.6676	0.7000	0.6806	0.6734
11.	Head length/Internarial distance	4.8540	4.7175	5.1623	5.3630
12.	Snout-vent length/Fore limb length	1.9611	1.8875	1.9635	1.8448
13.	Snout width/Snout length	1.8836	1.6546	1.8240	1.7509
14.	Inter narial distance/Inter Tympanum distance	0.2817	0.2898	0.2563	0.2685
15.	Head width/Inter Tympanum distance	1.3675	1.3672	1.4550	1.4402

the weight from 6.44 gm to 13.40 gm. The SV length of females varied 5.45 cm to 7.15 cm and the weight from 18.17 gm to 49.02 gm (Table 2.4). The graph plotted between SV length versus the corresponding weight of male and female frog separately and jointly showed a linear relationship LcCran (1951) expressed such relationship by a regression equation.

$$W = Cl^n$$

$$\text{Log } W = \log C + n \log L$$

where

W = weight of frog
L = length of frog
C = initial growth index
n = equilibrium constant

The constant of the above equations such as initial growth index 'C' and equilibrium constant 'n' have been found out from the slope and trend of the Figs. 2.15, 2.16 & 2.17 for males and females separately as well as together. The SV length and body weight of the male and female frogs were deduced in the formulae, to find out the initial growth index 'C' and equilibrium constant 'n'. The values of these constants were found to be same by (1) method of least square and (2) method

Table 2.4

Comparison between body weight taken and standard weight estimated as per the regression equation at given SV length of Rana cyanophlyctis males and females

S. No.	SV length (cm)	Weight taken (gm)	Weight estimated (gm)	SV length (cm)	Weight taken (gm)	Weight estimated (gm)
1.	4.05	7.42	6.79	5.45	18.17	18.66
2.	2.05	6.44	6.79	5.50	18.61	19.22
3.	4.10	7.60	7.13	5.55	18.78	19.80
4.	4.10	6.86	7.13	5.55	19.33	19.80
5.	4.10	7.46	7.13	5.60	18.56	20.38
6.	4.15	7.10	7.49	5.60	22.54	20.38
7.	4.15	6.96	7.49	5.65	21.46	20.99
8.	4.15	7.00	7.49	5.65	21.47	20.99
9.	4.20	8.72	7.86	5.70	23.88	21.60
10.	4.20	7.20	7.86	5.70	20.89	21.60
11.	4.20	6.70	7.86	5.70	22.95	21.60
12.	4.20	8.00	7.86	6.10	28.11	26.94
13.	4.20	7.40	7.86	6.10	25.35	26.94
14.	4.25	8.36	8.24	6.35	28.42	30.71
15.	4.30	11.70	8.64	6.50	36.28	33.13
16.	4.30	9.60	8.64	6.55	37.10	33.96
17.	4.30	6.95	8.64	6.60	34.96	34.82
18.	4.40	8.38	9.47	6.60	34.92	34.82
19.	4.40	8.80	9.47	6.75	34.17	37.46
20.	4.45	9.19	9.91	6.75	36.94	37.46
21.	4.45	12.01	9.91	6.80	37.17	38.37
22.	4.45	9.30	9.91	6.80	37.45	38.37
23.	4.45	10.28	9.91	6.85	45.05	39.30
24.	4.45	10.20	9.91	6.95	35.48	41.02
25.	4.50	11.63	10.37	6.95	38.36	41.02
26.	4.50	11.40	10.37	7.00	39.97	42.17

Table 2.4 continued

S. No.	SV length (cm)	Weight		SV length (cm)	Weight	
		taken (gm)	estimated (gm)		taken (gm)	estimated (gm)
27.	4.50	12.00	10.37	7.05	47.25	43.16
28.	4.65	12.60	11.83	7.05	45.63	43.16
29.	4.80	13.40	13.45	7.15	49.02	45.19
30.	4.90	12.90	14.61	7.15	45.53	45.19

Equation for Male

$$\log W = -1.6106 + 4.0214 \log L$$

$$W = 0.0245L^{4.0214}$$

Equation for Female

$$\log W = -1.1278 + 3.2575 \log L$$

$$W = 0.0745L^{3.2575}$$

where W = weight of the frog (gm)
L = length of the frog (cm)

Fig. 2.15 : Length-weight relationship in male populations of *Rana cyanophlyctis*

2.16 : Length-Weight relationship in female population of *Rana cyanophlyctis*

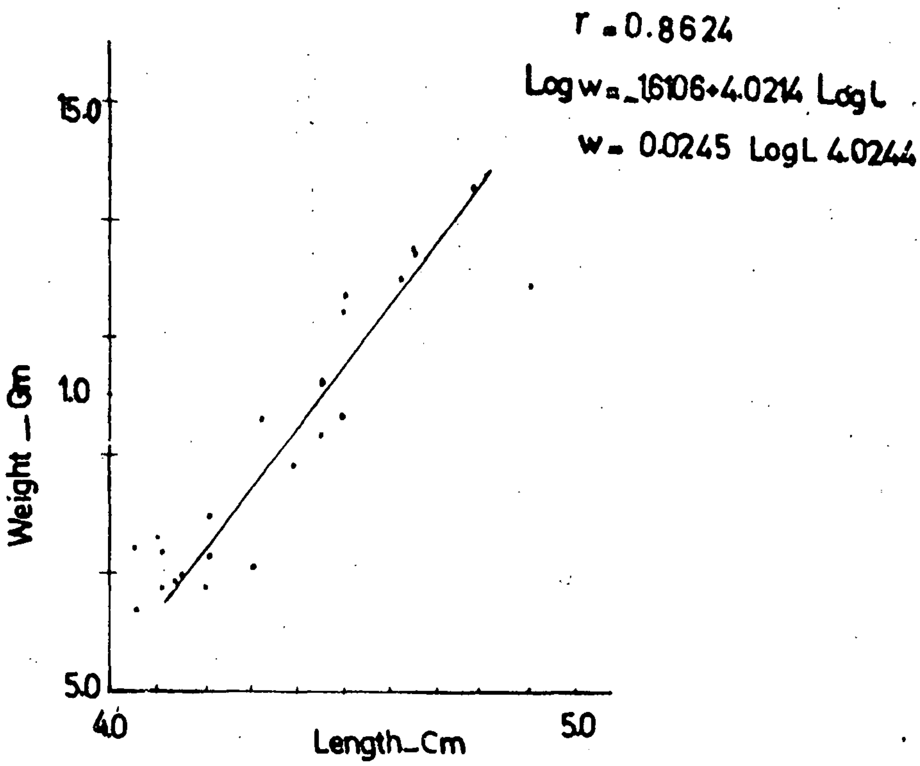

Fig. 2.15

Male

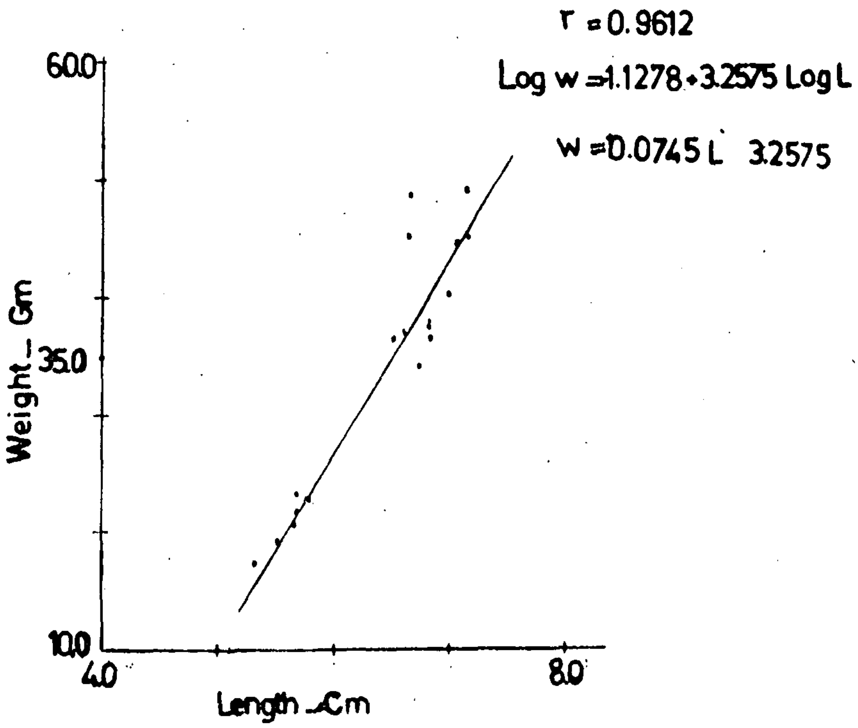

Fig. 2.16

of calculator programming. The initial growth index 'C' and equilibrium constant 'n' for males were found to be 0.0245 and 4.0214, for females 0.0745 and 3.2575 and for males and females jointly as 0.0422 and 3.125 respectively. Thus, the relationship between weight and length of the frogs were found to be as follows.

$$W = 0.0245L^{4.0214} \quad \text{for males}$$

$$W = 0.0745L^{3.2575} \quad \text{for females}$$

$$W = 0.0422L^{3.125} \quad \text{for combined frogs}$$

(See Figs. 2.15, 2.16 & 2.17)

Applying Peaksonian Product Movement equation the linear relationship and correlation coefficient has been calculated as under :

$$r = \frac{\sum XY}{n \; OX \cdot OY}$$

$$= \frac{\sum (X_1 - \bar{X})(Y_1 - \bar{Y})}{n \; OX \cdot OY}$$

where

X & Y = two variables (length and weight of the frog)

r = correlation coefficient

$\bar{Y}$ = arthematic mean of Y

$\bar{X}$ = arthematic mean of X

OX = standard deviation of variable 'X'

OY = standard deviation of variable 'Y'

Fig. 2.17 : Length-weight relationship in male and female taken together.

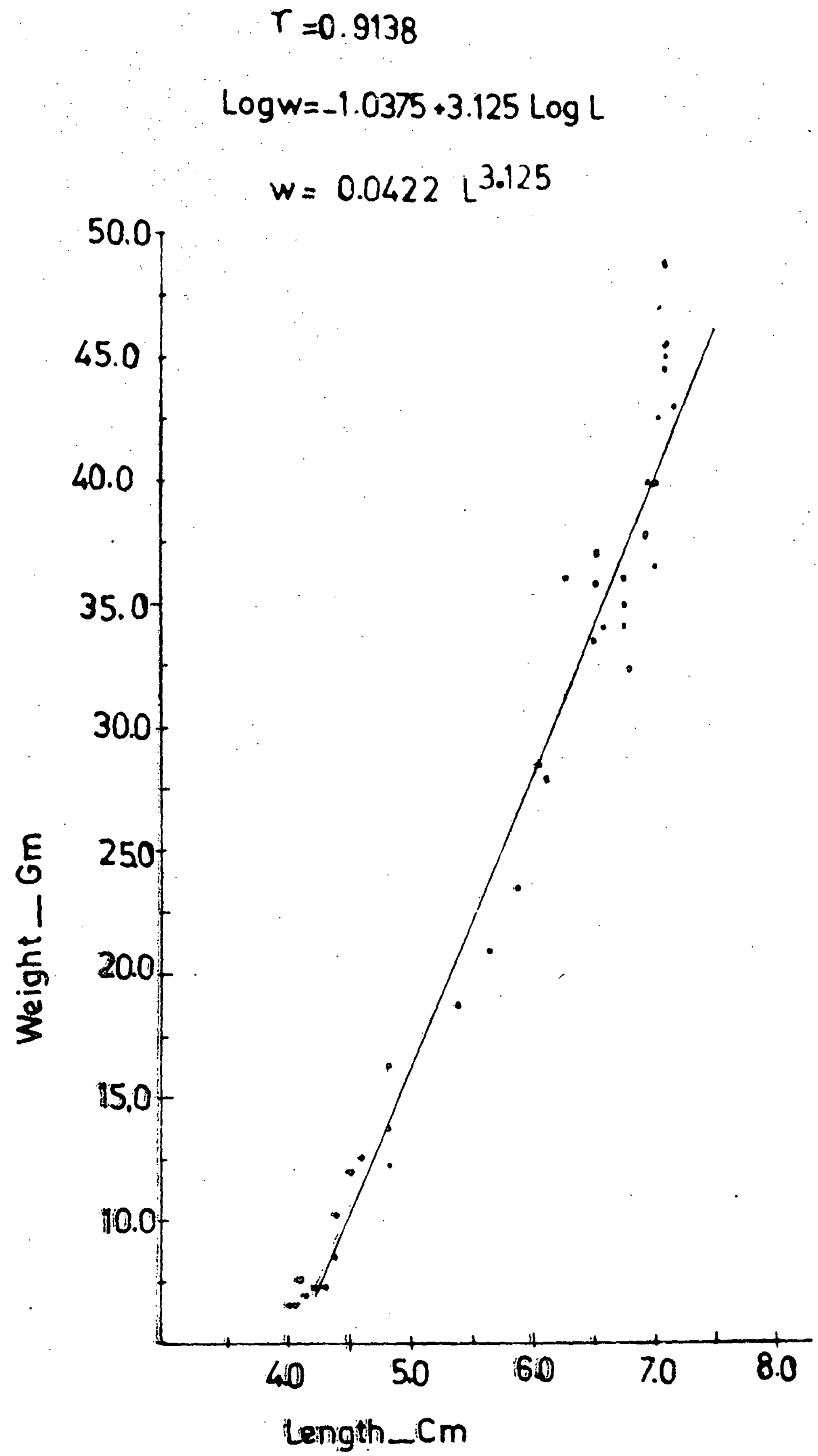
r =0.9138
Logw=-1.0375+3.125 Log L
w= 0.0422 L3.125
50.0
45.0
40.0
35.0
30.0
25.0
20.0
15.0
10.0
Weight__Gm
4.0
5.0
6.0
7.0
8.0
Length__Cm

Fig. 2.17

The bivariate distributions of length and weight in male and female frog separately and jointly were assessed for the degree of mutual relationship. The coefficient correlation of two variables, length and weight were found to be 0.8624, 0.9612 and 0.9138 respectively for male, female and male-females taken together respectively. The coefficient of correlations thus calculated were compared with the tabulated values and were found to be significant at 1% and 5% level of confidence.

The length of male and female frogs (Table 2.4) were applied to the derived "length and weight" relationship for formulae above, of male and female respectively, and thereafter the standard length and weight of each frog was calculated (Table 2.4). The measured weight and standard weight calculated were compared to find out the difference. The standard weight calculated represents ideal weight at given SVL for the frogs of Gauhati and Shillong population.

(b) <u>Relative condition:</u>

The relative condition, showing relationship between size and weight of the frog has been worked out for different seasons of the year. The adult frogs of

Table 2.5

Relative condition of Rana cyanophlyctis of Shillong and Gauhati make populations

Months	Length	Weight	Relative condition constant	Mean($\bar{X}$)	Standard deviation
Jan.	5.30 - 6.00	16.20 - 23.40	$0.1088 \times 10^5 - 0.1178 \times 10^5$	0.1126×10^5	0.0047×10^5
Feb.	4.40 - 6.30	14.10 - 28.95	$0.1132 \times 10^5 - 0.1514 \times 10^5$	0.1265×10^5	0.0170×10^5
Mar.	5.20 - 6.30	14.90 - 29.60	$0.1001 \times 10^5 - 0.1183 \times 10^5$	0.1111×10^5	0.00776×10^5
Apr.	5.20 - 5.90	16.35 - 24.52	$0.1161 \times 10^5 - 0.1307 \times 10^5$	0.1182×10^5	0.00691×10^5
May	6.60 - 6.80	34.94 - 37.45	$0.1201 \times 10^5 - 0.1349 \times 10^5$	0.1285×10^5	0.00671×10^5
Jun.	6.85 - 7.15	36.42 - 49.02	$0.1132 \times 10^5 - 0.1372 \times 10^5$	0.1299×10^5	0.0112×10^5
Jul.	6.60 - 6.95	34.16 - 38.36	$0.1078 \times 10^5 - 0.1247 \times 10^5$	0.1158×10^5	0.00745×10^5
Aug.	5.10 - 6.70	16.90 - 41.50	$0.1274 \times 10^5 - 0.1379 \times 10^5$	0.1326×10^5	0.0054×10^5
Sep.	5.10 - 5.60	12.80 - 15.45	$0.0892 \times 10^5 - 0.0965 \times 10^5$	0.0921×10^5	0.003×10^5
Oct.	5.40 - 5.90	14.95 - 16.70	$0.0813 \times 10^5 - 0.0949 \times 10^5$	0.0869×10^5	0.0059×10^5
Nov.	5.20 - 6.10	15.50 - 28.95	$0.1086 \times 10^5 - 0.1314 \times 10^5$	0.1197×10^5	0.0117×10^5
Dec.	4.50 - 5.85	10.34 - 22.65	$0.1117 \times 10^5 - 0.1167 \times 10^5$	0.1138×10^5	0.002×10^5

mixed sex with snout-vent length varying from 4.40 cm to 7.10 cm and weight 10.34 gm to 49.02 gm were used for the study. The average best relative condition during December was found to be 0.1326×10^5 in comparison to smallest size 0.0869×10^5 available during October. The range, mean and standard deviation of relative condition of frogs for different months have been shown in Table 2.5. The collection during June were predominated by large size and heavy female with mature ova. An ascending trend in the relative condition from March onwards has been recorded which reached its peak during June. June onward a decline in length and weight relationships have been noticed, reaching to its minimum during September and October. The second peak of robustness was observed during December. (Fig. 2.18). Table 2.6 shows correlation coefficient value of the relative condition of the frogs during different months with environmental factors like average atmospheric temperature, average aquatic temperature, humidity and rainfall of the Shillong site. Multiple correlation of all the above factors and relative condition of the frog have also been derived (Table 2.6). On 'F' test the correlation and multiple correlation have been found to be highly insignificant at 1% and 5% probabilities.

Annual Breeding Cycle

The frog does not hibernate and can be easily found in the vicinity of the water bodies throughout the year. Its annual breeding cycle does not show well demarcated phases as observed in the other terrestrial frogs. It has a prolonged breeding period. Based on availability of spawns, larvae, froglets, juveniles, adults and gravid females the annual breeding cycle of this species can be divided into 3 phases: (1) Pre breeding phase; (2) breeding phase and (3) post-breeding phase.

(1) Pre breeding phases:

The early breeding phase extends from mid-February till the end of April. The temperature during the period ranges from 8°C to 21°C and rainfall 0.0 to 84.4 mm. The population census in the first half of the period is marked by the predominance of juveniles. The second half period which is marked by an increase in atmospheric temperature from (6.8°C to 20°C) and in rainfall from (20.0 mm to 40.0 mm), shows an increase in the adult population on land. At the atmospheric temperature approximately 18°C and rainfall around (40.0 to 50.0 mm) the activities of male frog population are enhanced earlier than the female population. The population census of the frog on land at this stage reflects equal proportions of male and juvenile frogs. The activities in female frog enhances remarkably at the end of the phase, when temperature reaches above 20^{o}C and average rainfall around 100 mm.

During the last phase of this period (late April) a population of the female frog with enlarged abdomen and mature ovary are often encountered. In permanent water bodies amplexus can also be recorded. However, spawns and embryonic stages are not recorded. The induced breeding with the help of homoplastic pituitaries during this period is although a success, but produces small spawn size.

(2) Breeding phase:

Rana cyanophlyctis has an acyclic, prolonged breeding period. Its active breeding period extends from May and last till the early September. The period is marked by an increase in temperature (17^{o}C to 28^{o}C) and rainfall (120.0 to 460.0 mm). The noticeable feature of this period is increase in activities and abundance in frog population on land. The population structure assessed through random frog collection represents an almost equal number of males, females and juveniles. The large size female frogs often encountered in the period contain abdomen ladden by mature ova, however, their guts remain empty. Amplexus in nature are also observed in large number. Spawns and tadpoles of various developmental stages are recorded abundantly, both in slow running and stagnant permanent water bodies. In the present observation the maximum number of amplexus and spawns have been recorded in June, indicating a period when they are most actively envolved in breeding (temperature 22.0°C and average rainfall above 450 mm). A large number of froglets population are observed during September (temperature around 21°C and average rainfall around 250mm) probably a result of July spawning. Under laboratory condition the development from a fertilized ovum to a froglet requires more than two months. The induced breeding experiments performed were most successful and resulted into spawning of large number of ova.

(3) Post-breeding phase:

During the post-breeding phase the environmental conditions are marked by lowering in temperature from (25.5°C to 4.7°C) and rainfall (240.0 mm to 0.0 mm). With the decline in temperature and rainfall the relative abundance of frogs on land also shows a decreasing trend. This has been more marked in adult frog populations. The movement in adult population what so ever are restricted within or around water bodies. The juveniles and sub-adults however, remain active and are observed in abundance on land. At this period amplexus are rarely observed.

Moreover, large number of tadpoles of different developmental stages and sizes are recorded in abundance from the permanent water bodies. The females encountered at this stage often show few or reduced numbers of ova. Their guts are mostly filled with various types of food items. During warm evenings soon after rains, a marked increase in the abundance of various size group of frogs are recorded on land. Induced breeding at this stage although successful results in small spawn size.

Winter months at low temperatures (4.5°C to 15.0°C) and negligible rain, frogs are observed mostly restricted in the water bodies. Here adults and large size animals occupy the middle core and juveniles, the shallow edges of deep water bodies. The abundance of various size and sex of the frog on land has been marked at it lowest. The movement during cold months get reduced and restricted near the water bodies. During sunny hours frogs are observed basking and even floating in the water bodies (Fig. 3.4). Few large size tadpoles of advanced developmental stages can still be recorded till the middle of the December in permanent water bodies, along with juveniles and froglets which have been recently metamorphosed. Records of the breeding of the frogs in nature as well as with homoplastic pituitaries injections could not be achieved. No amplexus or spawn were observed during this period.

.... Contd. p. 54/-

DISCUSSION

Rana cyanophlyctis has been described since 16th century A.D. Daniel (1975) records that the ability of this species to skip over the surface of water like a ricocheting stone was remarked upon by the great Mughal Emperor Babar. The nomenclature, *Rana cyanophlyctis* was given by Schneider (1799) in History Amphibian Part I (see Boulenger, 1920). There are a number of references such as Thurston (1888); Boulenger (1890, 1920); Annandale (1904); Boulenger *et al.* (1907); McCann (1933, 1940, 1945); Narayan Rao (1915); Acharji and Kriplani (1951); Satyamurti (1967); Mohanty-Hejmadi (1974) and Daniel (1975). These descriptions are based mainly on the population available in the central, western and southern part of India. In the present investigation morphometry of its population available in the north-eastern part of India have been analysed. It would be noted that absolute measurements may vary depending upon the size of the animal but morphometric ratios are species specific.

(a) Absolute measurements:

(i) Male

Absolute measurements of 17 body parts of a sample of 20 frogs from Gauhati and 35 from Shillong have been presented in Tables 2.1 and 2.2. Boulenger

(1920) recorded largest males of Rana cyanophlyctis up to 6.6 cm from Aithalhim near Aden, 6.4 cm from South Arabia, and 4.5 cm from Travancore (India). In the present investigation the maximum SV length recorded for a male frog from Shillong was 5.3 cm and 5.1 cm from Gauhati. This size is larger than 4.4 cm recorded from western India (Daniel, 1975) and 4.2 cm recorded from South India (Satyamurti, 1967). Boulenger (1920) recorded that at Quetta the size of Rana cyanophlyctis collected in hills was larger than that of the plain ones. In the present finding also the Shillong hill frogs (1428 m a.s.l.) were found to be larger than Gauhati frogs(49.4 m a.s.l.) though certain morphological parts such as snout length, inter narial distance, tympanum and eye diameter were larger in Gauhati specimens. The size of head was large in Shillong frogs.

(ii) Female

Absolute measurements of 17 body parts of a sample of 34 frogs from Gauhati and 43 frogs from Shillong have been presented in Tables 2.1 & 2.2. The maximum SV length for female frogs from Shillong was 7.15 cm and 6.75 cm from Gauhati. However, Boulenger (1920) recorded 9.2 cm as maximum SV length of a female frog from Aithalhim near Aden. He reported largest SV length 6.5 cm from Kashmir (India), whereas Daniel (1975) reported approximately 6.0 cm from western India and Satyamurti (1967) recorded 6.2 cm from South India. All the measurements of female frogs were recorded to be larger for Shillong frogs than those of Gauhati frogs. This is in accordance to the Boulenger's (1920) observation at Quetta.

(b) Morphometric ratios:

Bragg (1965) while discussing the importance of ratio counts of morphological structures in intraspecific variation quotes that as far as a definition of difference between various forms are concerned, various well chosen ratios of different body parts provides values of much greater information. Thus, many anuran biologists of recent past such as Brown and Boschung (1954); Duellman and Klass (1964); Metter (1964); Heyer and Peters (1971); Tyler and Martin (1975); Tinsley (1973, 1975) and Roy (1979) have laid more emphasis on various ratio count studies than the absolute measurements. In the present investigation techniques of Cochran (1953) and Metter (1964) for estimating morphometric ratios among various body parts were followed. All together 15 ratio counts have been estimated for samples of frog populations of Gauhati and Shillong at 0.5 cm class interval. The table 2.3 contains the pooled data of the ratio counts for males (SV length range 5.3 to 4.0 cm for Shillong frogs and 5.1 to 4.0 cm for Gauhati frogs) and females (SV length range 7.15 to 5.15 cm for Shillong frogs and 6.75 to 5.00 cm for Gauhati frogs). The data for male and female frogs have been compiled in Tables 2.1 & 2.2. The ratio counts samples of the two population showed little difference. Further, the values obtained were subjected to student 't' test (see Chapter 5) and the differences were found to be insiginificant. Rodolfo (1957) reported that <u>Rana pipiens</u> collected from different latitudes and altitudes of Florida, Mexico and South Western United States showed little variation in the morphological measurements. However, they were not so major or significant to assign them a new race. Similarly, the <u>Rana cyanophlyctis</u> inhabiting Gauhati and Shillong do show little morphometric variation but not so significantly [illegible] (D_p^2 and Mean test analysis) so as to assign them a different race or strain.

(c) <u>Length-weight relationship</u>:

The length-weight relationships of <u>Rana cyanophlyctis</u> of samples collected from Gauhati and Shillong show a linear regression (Figs. 2.15, 2.16, 2.17). The correlation coefficient derived among the two variables of males and females frogs separately and in pooled conditions show significant 'r' values at both 1% and 5% confidence level.

Among anurans two types of the breeders have been recognised (1) Explosive breeder or single night breeder (2) Prolonged breeder or whole year breeder (Martof, 1953; Blair, 1968 and Wells, 1977). Rana cyanophlyctis shows continuous breeding habit in environmentally favourable localities (McCann, 1933; Ramaswami and Lakshman, 1959 and Gopalakrishnan and Rajasekarasetty, 1978). Gopalakrishnan and Rajasekarasetty noticed continous acyclic breeding in Rana cyanophlyctis and cyclic and seasonal breeding in Rana tigrina and Rana hexadactyla. In an explosive breeder, the weight of the frogs gets greatly reduced after its single night oviposition. Thus the SV length and weight, in such frogs does not show significant "correlation coefficient" in monthly samples or in the collection of whole year. It was noted that the weight of ova in mature and larger female constitute 1/5 of the total animal weight. Minimum record weight of the ovary was 1.71 gm and maximum was 11.59 gm. Further, the minimum number of ova was 1522 and the maximum was 6695 in Rana cyanophlyctis (see Table 7.10). It has been noted that Rana cyanophlyctis could be induced bred from March to October (see Chapter 6). However, the egg released on induced ovulation never exceeded 100 except during peak rainy seasons, when it was recorded maximum to be 667 ova. Further the spawn laid in nature hardly contains over 200 eggs (recorded by Ramaswami and Lakshman, 1959; Gopalakrishnan and Rajasekarasetty, 1977 and 1978). Hence, the maintenance of linear length-weight relationship in female throughout the year is attributed to a small spawn size.

Clarke (1974) and Labanick and Schlucter (1976) observed linear regression and correlation between body length and Tibia length of anurans. However, the constant of the regression equation and correlation coefficient which shows the trend of dependence and relationship was found varying. This indicates that the frogs of different species and different populations varies in the morphological and morphometrical relationship. However, the calculated weight and weight actually taken for samples of two population in the present investigation showed little difference. This suggests that the animals are identical and ideally placed at the two sites. From the regression equations derived, the length/weight relationships of the frogs at Shillong or Gauhati can be estimated if these measurements are known for any of the two populations.

Relative condition:

The relative condition is a relation of total length at a constant weight. The robustness, the bivariate and multivariate correlationship with annual environmental condition like atmospheric and aquatic temperature, humidity and rainfall did not showed much variations and had insignificant correlation coefficient (r = 0.29; 0.36; 0.20; 0.40 respectively and $P > 0.01$). Although annual fluctiaton in relative condition of the frogs have been observed varying little, the maximum robustness were recorded during May, June and July probably due to better maturity of the gonads. During this period the random collection of frogs from nature showed maximum number of large sized and heavy weight female frogs. The minimum relative conditions were observed during October and at this stage the random collection showed many light female with spent ovaries. From October onward an improvement in the relative condition (Fig. 2.18) seems to be associated with gonadal growth and post breeding ravenous feeding activities. The knowledge of relative condition of any frog in an annual estimation will provide a information of the robustness and subsequently the period when availability of heavy frogs of any size group are more. In <u>Rana cyanophlyctis</u> it is calculated to be May and June, when the availability of heavy frog should be maximum. This has also been recorded in population dynamics estimations (see Chapter 3). Hence, May–June collection of frog shall be an ideal for the procurement of healthy frogs for academic and economic purposes, including export to affluent countries as eatable items.

Annual breeding cycle:

Reproduction in the anuran can be divided into two categories (1) prolonged and (2) explosive. Wells (1977) recorded that prolonged breeding is probably more common although information on tropical species is so sketchy that generalization is hard to make. Many frogs in tropical region breed every month (see Church, 1960; Inger and Greenburg, 1963; Berry, 1964; Inger and Bacon, 1968; Brown and Alcala, 1970; Duellman, 1970; and Crump, 1974). The prolonged breeding activities in Indian anuran has not been worked out in detail although Daniel (1963, 1975) and Satyamurti (1967) have described anuran from western and southern part of India. McCann (1933) noted that *Rana cyanophlyctis* can be heard croaking in the permanent water bodies throughout the year, and under favourable condition it can also breed during winter months. In the present investigation it has been noted that the frog does not hibernate and can be easily found in the vicinity of permanent water bodies throughout the year at Shillong and Gauhati. Koskela (1975) in *Rana temporaria* and Roy (1979) in *Rana limnocharis* noted that their annual life cycle are divisible in two distinct phases (1) spawning, larval and active terrestrial phase and (2) wintering and hibernating phase . Koskela (1975) has further noted that *Rana temporaria* found inhabiting near water environment can be seen throughout the year near or within water bodies. In Rana cyanophlyctis, the annual breeding cycle does not shows demarcated phases as noted for terrestrial frogs. However, the annual periodicity can be observed in growth and behaviour of frog among its biological functions and environmental reactions. Based on the activities of the frog population and breeding pattern the annual cycle of Rana cyanophlyctis has been divided in three phases (1) early breeding, (2) breeding and (3) post breeding. The maximum activity in Rana cyanophlyctis population has been observed during breeding phase when temperature and rainfall both are recorded maximum. At low temperature and negligible rainfall the activities in the population gets greatly reduced. Further, the bivariate correlation coefficient observed between environmental conditions(temperature and rainfall) and activities also indicated high significant relationship (Table 3.4).

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SUMMARY

This chapter deals with an analysis of morphological characters, morphometric measurements (absolute as well as ratio counts) and length-weight relationship and annual breeding cycle of Gauhati and Shillong populations of Rana cyanophlyctis. This species is identified by somewhat broader than long depressed head, equal size of first and second fingers, slightly dilated and completely webbed toes and warty skin with pores and tubercles. Males are smaller and lighter (SVL 4.0 - 5.30 cm and body weight 7.0 - 19.0 gm) and females larger and heavier (SVL 5.10 - 7.20 cm and body weight 22.0 - 38.5 gm). Among the two populations, the frogs of Shillong population are larger and heavier than those of Gauhati population. However, the size of the head, internarial distance, diameter of the eye and tympanum of male frogs of Shillong were smaller than those of Gauhati frogs. Slight differences in certain ratio counts have been attributed to lentic and lotic habitats of the two places. There was a linear relationship between length and weight in the males ($r = 0.86$; $W = 0.0245\ L^{4.0214}$); females ($r = 0.96$; $W = 0.0745\ L^{3.2575}$) as well as males and females taken together ($r = 0.91$; $W = 0.0422\ L^{3.125}$). The relative condition of the frog did not show any marked variation.

The annual breeding cycle of the frogs is divisible into 3 phases: (i) Pre breeding (February to April), (ii) Breeding (May to September), (iii) Post breeding (October to January). They do not hibernate and are most active during breeding period. During winters their activity is at lowest pitch and at close vicinity of water bodies and are often seen basking in morning hours.

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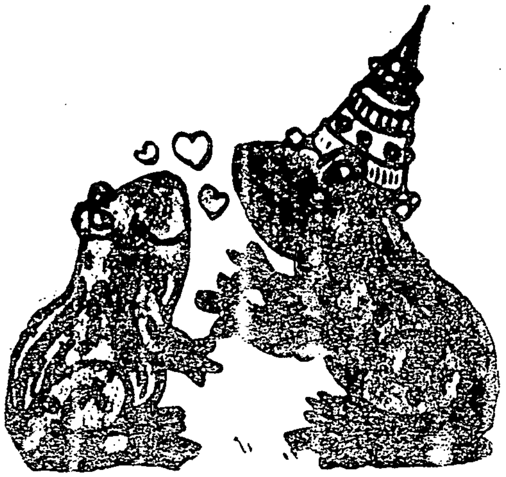
CHAPTER - 3

Relative abundance, Home range movement



INTRODUCTION

Clarke (1974) mentions that anurans have received less attention from ecologists than the other class of vertebrate, although they are extensively used for academic and economic purposes. Klopfer (1962) and Clarke (1974) noted that an understanding of anurans ecology can be useful because these animals have small behaviour repertoire than the higher vertebrate and a samll range of responses to environmental conditions. Hazlett et al. (1974) have also recorded the importance of animal study in natural environment. Goin and Goin (1962) have noticed that anurans exhibit basic life history features of all other vertebrates, right from the production of large number of youngs, with high initial mortality, to the bearing of few young in the adult form. Cole (1954) recorded that the different reproductive pattern of anurans have specific population consequences all of which can be elucidated, with proper knowledge of its environment and subsequent plastic behaviour of the animal.

The relative abundance and fluctuation in the anuran populations have been investigated by a number of workers (Jameson 1955, 1957; Ashby 1969; Zimka 1970; Koskela 1973; Pasanen and Koskela 1974; Koskela and Pasanen 1974). They have concentrated on various aspects of population dynamics such as its relation to reproduction, growth, migration and climatic conditions. In past few decades increasing attention has been paid to home site, home range and movement of anurans (see Martof 1953; Dole 1965, 1972, 1974; Calif 1973; Dole and Durant 1974). Dole and Durant (1974) also mentioned that anurans of tropical regions have not received that attention as of temperate regions. It is also true for *Rana cyanophlyctis* and other Indian anurans.

In the present chapter information on relative abundance, home range, movement and activities of populations of *Rana cyanophlyctis* available at Gauhati and Shillong in relation to environmental condition has been provided.

3.2 REVIEW OF LITERATURE

In past few years increasing attention is being paid to a better understanding of the population dynamics and behavioural responses of animals, particularly anurans. A survey of literature reveals that investigations on population dynamics of anurans have been restricted mainly to population fluctuation, relative density, movement and home range, and its relation to the environmental factors, such as rainfall, humidity and temperature.

A. <u>Estimation of relative population density:</u>

Various methods have been used to estimate the relative population density of insects (see Llcyd and Johnson, 1927; Potts, 1930; Cause, 1932; Ford and Ford, 1930; Nash, 1933; Jackson, 1933, 1936, 1939, 1940, 1944, 1948; Williams, 1940; Davidson and Andrewartha, 1948a,b; Johnson, 1950, 1950, 1952, 1955; Banks, 1954; Andrewartha and Birch, 1954; Bnowning, 1959 and Andrewartha, 1957, 1961) but there are relatively few references on larger animals. Lincoln (1930) used 'Lincoln Index' to estimate the population in ducks. Jackson (1936) modified the Lincoln's technique and propounded a new technique mamely capture, marked release and recapture technique. This technique has been assisted by various statistical equations to compensate the small size population, death, birth survivalship immigration, migration, mobility and immobility in the population (see Haynes, 1949; Bailey, 1951; Leslie and Chitty, 1951; Leslie, 1952; Chapman, 1952; Boguslavsky, 1956; Dorroch, 1959; Jolly, 1965; Cormack, 1964; Manly, 1970; 1971, 1973, 1974 and 1977; Martin, 1970; Iwo, 1971; Ito, 1973; Carothers, 1973; Ito et al. 1974; Hamada, 1976; Kundo, 1977). The Jackson's (1936) capture, marked release recapture methods have been successfully used in wide range of animals such as Lepidoptera Polyommatus iracus (Dowdeswell et al. 1940; Dowdeswell, 1952), Mus musculus (Evens, 1949), fishes (Greking, 1952), birds (Manly, 1977), Dacus cucurbitae (Ito et al., 1974), rats (Leslie and Davis, 1939) water voles: rodents (Worrel, 1964; Zijda, 1971,1972; Stoddart, 1970; Gaisler and Zejda, 1973 a,b; Holisova and Pelikan, 1974 and Airoldi, 1976) and frogs (Jameson, 1955, 1957).

Among amphibians the capture marked release and recapture technique has been employed to study the home range, seasonal activities, movements and population fluctuations in different frog populations (see Dole, 1965, 1974; Martof, 1953 a,b, 1956; Fitch, 1956, 1958; Dole and Durant, 1974; Clarke, 1974; Krikorian, 1976, 1977; Berra and Gunning, 1972; and Currie and Bellis, 1969). Dole and his group have investigated home range movements of Hyla cadaverina , spatial relations and home range of natural population of Rana pipiens, homing and orientation of *Bufo americanus* and home site movement and seasonal activity of *Atelopus exyrhynchus*. Other investigations on population dynamics of anurans have been made by Martof (1953) on *Rana clamitans*, Koskela and Pasanen (1974) on *Rana temporaria*, Clarke (1974) on *Bufo*; Bellis (1965) on *Rana sylvistica*, Jameson (1955) on *Syrrhophus manocke* and Currie and Bellis (1969) on *Rana catesbeiana* . The technique of catch per time, to estimate the basolute density of the populations at various sites has been applied by Koskela and Pasanen (1974, 1975) on *Rana temporaria* , Currie and Bellis (1969) on *Rana catesbeiana* , Hazlett *et al*. (1974) on crayfish *Orconectes virilis* Zejada (1972) on water vole.

Home Range:

Home range ecology of anurans may be elaborated in detail. Burt (1943) defined home range an area usually around the home site over which the animal normally travels in search of food and excluding occasional movements outside the area, perhaps of its exploratory nature. Kelby (1945) discussed that competition for food has been a major factor for regulating home range in anurans. In species, where competition are greater, the activity area and home range are larger.

The region of movement during daily activity around home site has been termed as "active range" (Carpenter, 1952) and was observed to be varying with the size of the animal. Dole and Durant (1965, 1974) defined home range as a place where animal returns again and again. Dole (1965) observed that home range size in anurans are often positively correlated to its body length, however, the frog collected from Budzinski's had no statistical significant correlations between body length and home size further, he noted that anuran home range are dependent on factors like food, shelter and environment condition, acting singly or in continuation.

Commenting on the behaviour of Rana cyanophlyctis McCann (1933) observed they are extremely active creature and much at ease at land as well as water. During day time it rarely stays far from the water bodies almost except during monsoon months when it can be found almost anywhere. There appears to be no doubt that this species does undertake long journey during night or else it would be difficult to account for its sudden appearance in water holes which have been recently excavated. Grabman (1950) pointed out that the natural movement range does not include all points from where animals are captured. The movement are often observed to be governed by numerous external factor and availability of food and shelter. Observing the home range in agnetic frog, Martof (1953) found that juveniles and small size frog occupied the shallow portion of water and adult and large size the central deep water. The home range of Syrrhophus marnock (Jameson 1955) was found to be dependent with the availability of the shelter area. Jameson (1956) observed that spadefoot toad and strecke chorus frog marked in previous year returned to their home site during three successive years. Jameson (1957) mentioned that male frog and toads reached the breeding site first and subadult and female followed afterwards only after summer rain. In Pacific tree frog he recorded that males, females and subadult move approximately 1000 yards to reach the home site. Willis et al. (1956) marked the frogs to observe its movement, they recorded that the frogs have affinity for home and returning back after daily routine activity. Based on marked, release, recacpture observation Bellis (1959) noted that the toad Bufo and Rana sylvatica have home areaduring summer months. However, Poor recapture in successive years for the same site indicates either high mortality or changing site every year. Through capture, marked, release and recaptured data Zejda (1971) noted that the size and sex ratios in the capture, numbers of individual captured maximum time, correlation of animal capture and its body size, relation between size group and distance covered by the male and female do not have any definite tren in water vole. Clarke (1974) observed that after 7 months of dormancy *Bufo woodhousei* starts its reproduction activity which declined with lapse of time and noted that marked toad used the same home for 2-3 successive years and its home range varied from 21.8 m to 32.3 m. Further, the activity and movement of *Bufo woodhousei* has been observed as bimodel type with maximum activity at dusky and dawn hour i.e. morning sun rise and evening sun set hour.

Contribution on the population dynamics in relation to environmental factors can be described as follows.

Humidity and rainfall:

McCann (1933) observed that a large number of frogs and toads found in India are nocturnal, though during rainy season and at wet localities they become diurnal and nocturnal both. He described *Rana cyanophlyctis* as nocturnal frog capable of long journey depending upon the rainfall and humidity. Blair (1953) observed an aggregation of 357 mexican toad *Bufo vellicaps* of a varying sizes ranging from 10 mm to 55 mm and noted that the aggregation broke on rainy days, indicating that the rains excite the toads to move away and terminate the hibernation. Describing the movement of the pacific tree frog, Jameson (1959) noted that rains of warmer spring bring about termination of wintering and movement of the female hylid frogs and leads its movement to breeding sites. Amorono and Marshall (1960) mentioned that rainfall and temperature and various other external factors (stimuli) jointly induce the reproductive behaviour in anurans. Dole (1963) observed that the movement of the frog antelops depend upon sources of water, soil moisture and dew more than food.

While investigating the behaviour of self maintaining population of *Rana temporaria* Ashby (1969) and Koskela (1973) noted that heavy rains during warm weathers activate the frogs movements and breeding. Koskela attributed this movement to rains more than sunshine ($x^2 = 225$ P 0.001). Heyer and Bellin (1973) observed significant correlation between heavy rain and mating success; heavy rain and larval success and heavy rain and mating calls in leptodactylid frogs and recorded that leptodactylids are probably the best colonizer species and are well adopted to changing environmental conditions. Koskela (1973) observed that *Rana temporaria* becomes in active for two hours during mid day and at bright sunshine. He reported maximum breeding migration of the frog at 84% mean air humidity. Martof (1953) in *Rana clamitans* and Currie and Bellis (1974) in

Rana catesbeiana recorded maximum activity in the population during warm, rainy night than on any other climatic condition.

Temperature:

So far as temperature is concerned Stuart (1951) described two types of temperature thresholds effecting the availability and behaviour of anurans:

(1) Critical minimum - The temperature at which animals becomes helpless to escape enemies or to remedy their thermal impass.

(2) Critical maximum - The thermal point at which locomotary activities become disorganized and the animal cannot escape and this promptly leads to death in animal.

Discussing the role of temperature and the availability of *Bufo* at different elevations Stuart reported that *Bufo marinus* was not found above 1500 m, however, its critical minimum temperature (15°C) was recorded 3.5°C below than any other toad available at lower elevations. The difference in the critical maximum temperature of *B.bocusta* and *B. marinus* has been noted as an effective reason for the restriction of the above toads at different elevations and localities. He, further remarked that zoogeographic distributions of any anuran species with respiratory intake or haemoglobin counting or temperature have no correlation. McCann (1933) noted that on sudden fluctuations in the environmental temperature anuran species found in India including Rana cyanophlyctis move to the middle core of water bodies. Mullally (1952) observed that the toad Bufo boreas was found to be active throughout the year at night till the temperature was recorded above 3°C below which it solidifies and dies. Many anuran species are active at high temperature (Martof, 1953; Hensen, 1957; Jameson, 1957; Ashby, 1969; Currie and Bellis, 1969, [illegible]). At extreme hot or cold period they hide in holes under stone or in moist soil or migrate deep into water bodies (see Currie and Bellis, 1969, 1974; Koskela, 1973; Koskela and Pasanen, 1975; Bohmsack, 1961 and Dole and Durant, 1974).

Commenting upon the importance of temperature in diurnal behaviour Fitch (1956) and Hadfield (1966) mentioned that actodermal basking, in anurans, up to certain optimum temperature enhances metabolic activities and help in digestion and facilitation. Martof (1953) in Rana clamitans, Hansen (1955) in Rana heckschoni have also recorded basking at the edges of water bodies having moist surface.

In some amphibians and reptiles (Turner and Hopkin, 1972; Faber, 1974; Krekorien, 1976), the basking sites are reported to be depended and guarded by male populations.

In 1975, Koskela and Pasanen reported that the change in weight of females was inversely proportional to the weight of ovaries, and due to its water content it increases by 97% during winter month.

In India, Gopalakrishnan and Rajasekharasetty (1977, 1978) work on the annual reproductive activity of internal hypophysio-gonadal rythm in Rana hexadactyla and Rana tigrina and reported that it was regulated by atmosphere temperature, relative humidity and rainfall.

Limnological Studies:

For limnological studies workers like Burger (1950) in spadefoot toad; Ruibal (1955) and Rubin (1959) in Rana pipiens; Metter (1961) in Bufo boreas; Fingal and Kaplan (1963) in Xenopus; Crooke (1975) in Bufo bufo and Rana temporaria; Beebee and Graffin (1977) in Bufo colamita; Bufo bufo and Rana clamitans and Saber and

Dunson (1978) on Rana catesbeiana, have analysed factors such as pH; conductivity; phosphate; calcium; carbonate; bicarbonate; chloride; total solid particles; dissolve solid particle; suspended solid particles, salinity and iron and carbon minerals to find out the relationship in limnological conditions with reproductive behaviour and developmental rate of anuran tadpoles and adults.

3.3. MATERIALS AND METHODS

The relative abundance, home range, movement and behaviour of the populations of Rana cyanophlyctis (Schneider) have been investigated at Gauhati. At Shillong relative abundance was estimated with a view to compare it with that of Gauhati.

At Gauhati, the collections were made during dusky hours of evening or at night, fortnightly throughout the year 1978.

The collection at Umkhrah river site at Shillong were made fortnightly throughout the year 1978, during 'day time', as it was not safe to visit the remote site area at night due to difficult hilly terrain. In view of the limitations at the sites Gauhati and Shillong the searches during day hours and night hours respectively were made only when it was believed that information not otherwise obtainable might be secured.

The availability of the males, females and juveniles and their numbers of catch per hour was estimated monthly on the numbers of frog collected in 3 hours throughout the year 1978. The number of catches of males, females, and juveniles per hour, was correlated with physical and chemical conditions of the sites.

For the behavioural study a census was made by walking slowly back and forth across the site area systematically covering all the area. The intensive search was made at the vicinity of water bodies, as the animals mostly confined themself near these except on the occasion of feeding and breeding, when they travel at large to meet the requirement. During census, the frogs were collected by hand or by an insect net, made of strong thread net attached to an iron road.

The slow and cautious walking at the Gauhati study site, at dark nights, does not seem to disturb the anurans. They were readily seen by a torchlight, which dazed and rendered them immobile, and easily captured. At Shillong animal were approached from behind and then caught by sudden, quick, swinging flush of net. The escaped and hiding frogs were collected by digging and sieving the mud of the hiding area by an insect net.

The technique of Krekorian (1976) to use nail polish and oil-paints did not prove successful for marking the animals as the colours either faded or were washed out after sometime. As such, marking and recognition by toe clipping technique of Martof (1953) was followed. The toes of each frog were clipped. Such frogs mostly confined themselves in water and avoided skipping or floating on surface. These difficulties were overcome by the use of different coloured threads which were tied round the thighs. The various colour combinations and numbers of folds of the thread ensured identification of the animal on recapture.

The population of the animal at Ulubari Fish Farm Gauhati was estimated by Jackson's (1939) technique of capture, marked, released and recapture at monthly intervals throughout the year 1978. The following formula to estimate their density have been used :

$$\frac{\text{Number marked individual recorded in census 'N'}}{\text{Number individual marked in pre-census 'X'}} = \frac{\text{Number individuals unmarked obtained in census}}{\text{'P' Total number of marked individual at end of precensus}}$$

(It has also been used by Jameson (1957) for the estimation of population structure and homing in pacific tree frog).

Hence,

Total number = P + X

Total population (Population estimated + Individual marked in precensus)

The animals were captured, mostly by hand or by net, during night hours. The animals thus captured were marked and released always at the junction of the nursery pond No.1, 2, 7 and 8 (Fig. No. 1.1.1). The frogs were captured marked and released again 20-24 hours after the previous release. For every set of experiment the frogs were captured two times and released four times. After each capture the frogs were marked with different colour threads. Data on each frog was recorded at each capture: the colouration, the place of capture, numbers of frogs caught per hour, sex ratio, size group behaviour their movements from the release site were routinely recorded. The presence or absence of the vocal sac was noted to distinguish males from females. The male frogs of snout vent length below 3.8 cm and females of SV length below 5.70 cm were considered as sub-adult and juveniles. Records of the abundance of various size group, at 1.0 cm class interval and the total members of juveniles, males and female in a population at each such forays were made. Mode of frog size (SV length) in a population that occurs most frequent and around which size group maximum number of observations are concentrated and median i.e. the location of the median value that divides the population structure in two equal half were found out during each census.

The maximum and minimum air temperature, water temperature humidity, rainfall were regularly recorded. Climatic data were also cross checked from the data collected from the Meteorological Centres at Shillong and Gauhati. The pH, conductivity, carbonate, bicarbonates, chlorides, calcium and magnesium of water samples of the study sites were analysed once in the middle every month. The pH of the samples was determined by E.Lico, pH meter model 4-10, at the sample temperature. The sensitivity of the instrument was adjusted by using known buffer of 4.00, 7.00, 9.20 pH value. The least count of the instrument was 0.05. The other chemical factors were analysed as per the technique described by APHA (1955), Welch (1952) and Honda (1974).

Conductivity - The conductivity of the samples were measured, monthly by systronic direct reading conductivity meter 303 Sl.No.109. For standardization 0.01N KOH solution was used. The instrument was set at the sample temperature and equivalent standard value (calculated through standard graph) to obtain the conductivity of the samples directly in micromhos per mm.

Carbonate - The carbonate of the samples were analysed by Acid-Base titration technique. The samples having less than 8.3 pH are reported to have carbonate beyond estimation, that means there is no free carbonate in water sample. The carbonate from the water samples having pH more than 8.3 were calculated by titrating 20 ml samples with N/50 H_2SO_4 ; 2-3 drops of phenolpthalelin was used as an indicator, the end point being a point where the sample solution become colourless. The volume of the acid consumed was noted and multiplied by 30 equivalent weight of carbonate to know the carbonate in the samples in ppm.

Bicarbonate - The bicarbonate was estimated by titrating 20 sample water with N/50 H_2SO_4 solutions. Mixed solution (0.2 gm methylred and 0.01 gm Bromocresol green in 1000 ml of 95% Ethyl alcohal) was used as mixed indicator. The end point was taken at the point where 20 ml sample solution turned light pink. The bicarbonate was calculated in ppm by multiplying the end point (Volume of N/50 H_2SO_4 consumed) to 61.01 (equivalent weight of bicarbonate).

Chloride - The chlorides were calculated by Mohr's method. To a 20 ml water sample 4 drops of K_2CrO_4 as indicator solution (5.0 gm, dissolved in 100 ml chlorine free water) were added and then titrated with N/50 silver-nitrate solution, till the solution turned pink red. The volume of N/50 $AgNO_3$ consumed for titration was recorded, and have multiplied by equivalent weight of chloride 35.46 to estimate the chloride in the sample water, in ppm.

Total hardness - Total hardness of the water samples was calculated by titration. To a 20 ml of each water sample 2-3 ml of ammonia buffers were added till the solution gave smell of NH_3 . To this 5 drops of E.B.T. indicator (Eriochrome black. T.) were added, finally the above solution was titrated with N/50 standard solution of E.D.T.A. (Ethaline, diamine, titracitic acid solution) till the solution gave blue colour, which is the end point. The volume of E.D.T.A. solutions consumed during titration was equivalent to the ions for total hardness and hence was multiplied by 50 (factor for total hardness) to know the total hardness of the water samples. NH_3 buffer was prepared by dissolving 35.0 gm of NH_4Cl in 284 ml of NH_4OH of specific gravity 0.88 and then the solution was diluted to 500 ml . E.B.T. indicator was prepared by dissolving 0.05 gm of E.B.T. in 90 ml of Triethonal amine to the solutions added 10 ml of methanol so that the solution become less viscuss.

Calcium - E.D.T. solution was prepared by dissolving 3.72 gm sodium ethyl diamine tetra accetate (dried already at 80°C or over H_2SO_4 in a dissicator for one night) in 100 ml deionized water. The calcium of the water was estimated by adding 3 ml of N sodium hydroxide solution in 20 ml of water samples and 0.01 gm of pattern and reader reagent as indicator. The above solutions was titrated with N/50 E.D.T.A. solution till a violet colour was reached, which is the end point. The value of E.D.T.A. consumed (multiplied by equivalent weight 20.04) gives the calcium in ppm.

Magnesium - The magnesium from water samples was calculated by difference. From the measured total hardness (ppm) of water the analysed ppm value of calcium is substracted to achieve the magnesium of the water sample in ppm

A multiple correlation of the above mention factors was also calculated in the juvenile male and female population at the two sites.

5.4 OBSERVATIONS

12 months relative abundance by catch per hour, and capture, marked, release and recapture techniques and home range movement were investigated.

5.4.1 Relative abundance:

The relative abundance of the Rana cyanophlyctis on land, at two sites have been investigated from January to December 1978.

(a) Catch unit hour

The availability and fluctuation in the population of Rana cyanophlyctis was estimated at monthly intervals during 1978. The frogs were collected at random, every time for a minimum of three hours or more, and then number of frog per hour was estimated. To maintain the unformity and minimize the error following precautions were taken:

(1) In all census the procedure described were strictly adhered to, and the collection were made by visiting throughout the study site.

(2) Dawn and dusky hour were kept as the time for collection during every census.

GAUHATI POPULATION

The monthly relative abundance through catch/hour from January to December 1978 was estimated as follows. The data has been compiled and presented in Table 3.1 Fig. 3.1

January - Census was made on 12.1.1978. The atmospheric temperature was 12.0°C and humidity 82%. 21 frogs (3 males, 1 females and 17 juveniles) were collected in 4.0 hour. The SVL of the frogs in the sample ranged from 3.3 cm to 5.5 cm. The median SVL being 4.5 cm and showed the mode of 4.2 cm, showing the predominance of juveniles. Aggregation of the frog population in pond water and basking on sunny days were also observed.

February - Census was made on 9.2.1978. The atmospheric temperature was 15.5°C and humidity 71%. 20 frogs (7 males, 6 female and 7 juveniles) were collected in 3.00 hours. The SVL of the frogs in the sample ranged from 4.00 cm to 6.2 cm. The median SVL being 5.1 cm and showed a mode of 5.5 cm. The frog population was restricted mainly near the water bodies.

March - Census was made on 10.3.1978. The atmospheric temperature was 22.0°C and humidity 69%. 40 frogs (18 males, 8 female and 14 juveniles) were collected in 5.0 hours. The SVL of the frogs in the sample ranged from 3.5 cm to 6.2 cm. The median SVL being 4.8 cm and had two modes of 4.4 and 4.8 cm. The relative abundance of male has been maximum. The movement and active range has further increased.

Table 3.1

Catch/hour density of frogs at Ulubari, Gauhati

Date	Temp °C	Humidity %	Number of frogs collected	Duration		Snout vent length size group 1.0 cm class interval					Median	Mode	Catch per hour	Sex ratio M:F:J
				hr	min	2.00 2.90	3.00 3.90	4.00 4.90	5.00 5.90	6.00 above				
2. 1.78	12.0	82	21	4	0	1	5	9	6	-	4.5	4.2	5	3:1:17
9. 2.78	15.5	71	20	3	0	-	-	9	9	2	5.1	5.5	6.5	7:6:7
10. 3.78	22.0	69	40	5	0	-	2	24	10	4	4.8	4.4	8	9:4:7
7. 4.78	24.5	81	40	4	0	-	4	20	10	6	4.7	4.7	10	7:6:7
10. 5.78	25.5	75	40	3	0	-	6	16	12	6	4.6	4.6	13	9:5:6
11. 6.78	29.0	95	42	3	30	-	-	12	22	8	5.4	5.5	12	5:10:6
16. 7.78	30.5	83	40	3	10	-	2	20	10	8	4.7	4.2	13	5:3:2
10. 8.78	31.0	98	52	4	0	-	2	16	22	12	5.3	5.9	12.5	11:11:4
19. 9.78	31.0	81	26	3	0	-	-	14	16	5	4.6	4.6	9	5:4:4
6.10.78	30.3	82	40	5	0	-	2	18	14	6	4.9	4.6	8	6:7:7
8.10.78	23.0	87	38	5	0	-	8	14	12	4	4.6	4.3	7.5	5:3:11
8.12.78	18.0	86	21	3	0	1	8	5	6	1	4.7	4.2	7	3:3:15

<u>April</u> - Census was made on 7.4.1978. The atmospheric temperature was 24.5°C and humidity 81%. 40 frogs (14 males, 12 female and 14 juveniles) were collected in 4.0 hours. The SVL of the frogs in the sample ranged from 3.5 cm to 6.2 cm. The median SVL being 4.7 cm and showed the mode of 4.7 cm. The relative abundance of the female frog has increased in the population.

<u>May</u> - Census was made on 10.5.1978. The atmospheric temperature was 25.5°C and humidity 75%. 40 frogs (18 males, 10 females and 12 juveniles) were collected in 3.0 hours. The SVL of the frogs in the sample ranged from 3.6 cm to 6.2 cm. The median SVL being 4.6 cm and showed the mode of 4.6 cm. The relative abundance of the different size group (SVL) in the population has been high. It further, increased on rains.

<u>June</u> - Census was made on 11.6.1978. The atmospheric temperature was 29°C and humidity 95%. 42 frogs (10 males, 20 females and 12 juveniles) were collected in 3 hours 30 minutes. The SVL of the frogs in the sample ranged from 4.0 cm to 6.5 cm. The median SVL being 5.4 cm and showed the mode of 5.5 cm. The abundance of the frog population on dark rainy night which also had some drizzles showed maximum and subsequently maximum catch/hr can be made. The relative abundance of the female frog has been maximum.

<u>July</u> - Census was made on 16.7.1978. The atmospheric temperature was 30.5°C and humidity 83%. 40 frogs (20 males, 12 females and 8 juveniles) were collected in 3 hours 10 minutes. The SVL of the frogs in the sample ranged from 3.9 cm to 6.2 cm. The median SVL being 4.7 cm and showed the mode of 4.2 cm. The relative abundance on land has been predominated by adult frog.

<u>August</u> - Census was made on 10.8.1978. The atmospheric temperature was 31°C and humidity 98%. 52 frogs (22 males, 22 females and 8 juveniles) were collected in 4.0 hours. The SVL of the frogs in the sample ranged from 3.9 cm to 6.7 cm. The median SVL being 4.6 cm and showed the mode of 4.6 cm. The relative abundance of the adult frog were recorded maximum.

<u>September</u> - Census was made on 19.9.1978. The atmospheric temperature was 31°C and humidity 81%. 26 frogs (10 males, 8 females and 8 juveniles) were collected in 3.00 hours. The SVL of the frogs in sample ranged from 3.9 cm to 6.7 cm. The median SVL being 4.6 cm and showed the mode of 4.6 cm. The relative abundance of the frog on land showed a decreasing trend than August.

<u>October</u> - Census was made on 6.10.1978. The atmospheric temperature was 30.3°C and humidity 82%. 40 frogs (12 males, 14 females and 14 juveniles) were collected in 5.0 hours. The SVL of the frogs in the sample ranged from 3.7 cm to 6.3 cm. The median SVL being 4.9 cm and showed the mode of 4.6 cm.

Fig. 3.1 : Availability of Rana cyanophlyctis in relation to environmental conditions during different months of 1978 at Gauhati Site.

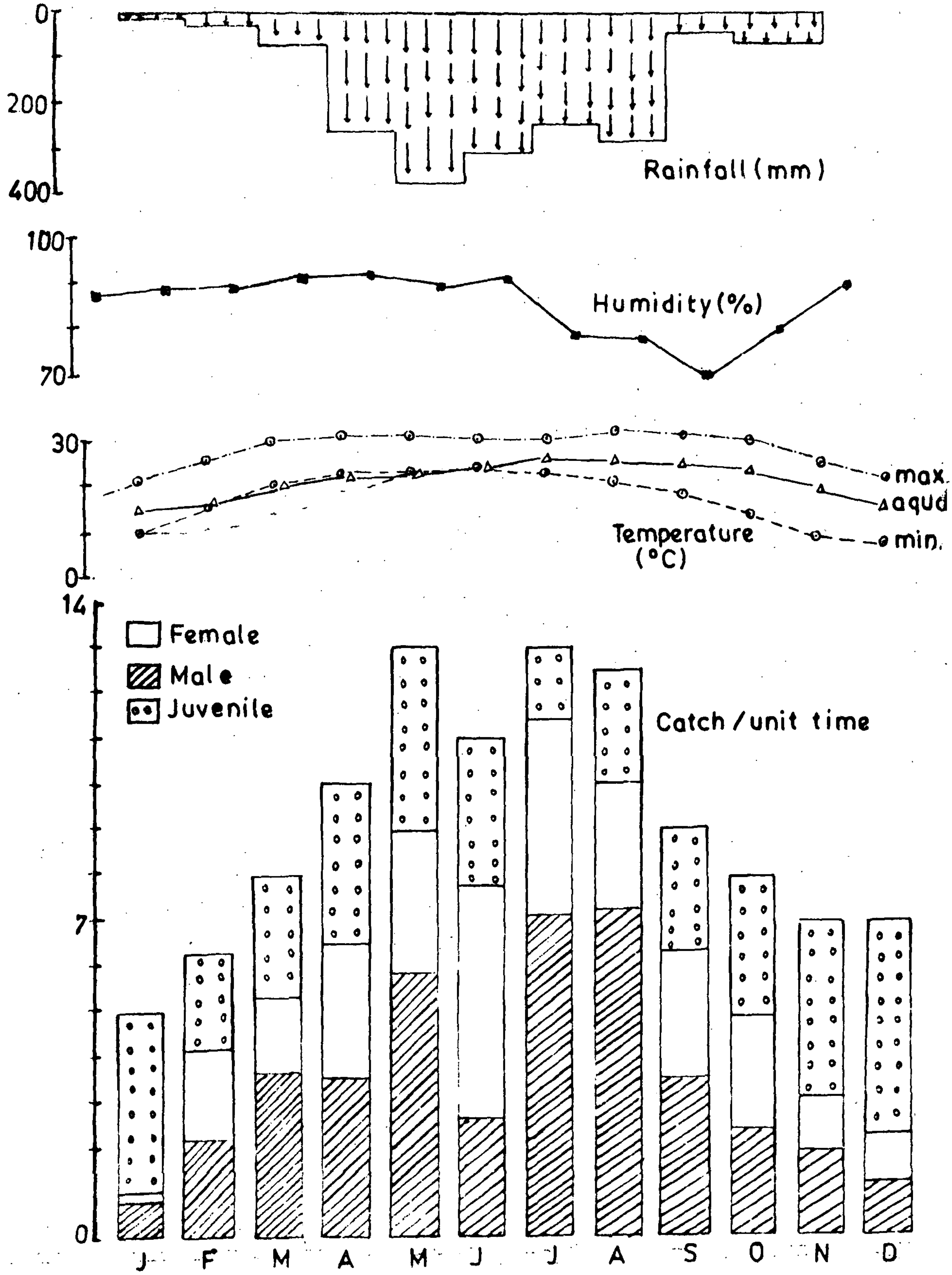

0
200
400
Rainfall (mm)
100
70
Humidity (%)
30
0
max.
aqud
Temperature (°C)
min.
14
7
0
Female
Male
Juvenile
Catch / unit time
J
F
M
A
M
J
J
A
S
O
N
D

Fig. 3.1

<u>November</u> - Census was made on 8.11.1978. The atmospheric temperature was 23.0°C and humidity 87%. 38 frogs (10 males, 6 females and 22 juveniles) were collected in 5.0 hours. The SVL of the frogs in the sample ranged from 3.4 cm to 6.3 cm. The median SVL being 4.6 cm and showed the mode of 4.3 cm. The relative abundance of the adult frog population on land has decreased. The junveniles prodominate movement and availability on land.

<u>December</u> - Census was made on 8.12.1978. The atmospheric temperature was 17°C and humidity 86%. 21 frogs (3 males, 3 females and 15 juveniles) were collected in 3.0 hours. The SVL of the frogs in the sample ranged from 3.4 cm to 6.0 cm. The median SVL being 4.7 cm and showed the mode of 4.2 cm. The frog populations are found more in water bodies, than on land. Adult occupying central deep portion and junveniles and subadult the shallow edges. The movement and active range reduced and restricted near the water bodies.

SHILLONG POPULATION

The monthly relative abundance, through catch/hour was estimated from Janyary to December 1978. The data has been compiled and presented in Table 3.2 ; Fig.3.2 . The estimation were as follows:

<u>January</u> - Census was made on 5.1.78. The atmospheric temperature was 80°C and humidity 70%. 20 frogs (1 male, 0 female and 19 juveniles) were collected in 5 hours. The SVL of the frogs in the sample ranged from 2.0 cm to 5.4 cm. The median SVL being 2.8 cm and showed a mode of 2.8 cm, showing the predominance of juveniles.

<u>February</u> - Census was made on 24.2.78. The atmospheric temperature was 11.5°C and humidity 65%. 22 frogs (2 males, 1 female and 19 juveniles) were collected in 4 hours. The SVL of the frogs in the sample ranged from 2.6 cm to 5.9 cm. The median SVL being 3.7 cm and showed two modes of 3.1, 3.4 cm, showing the predominance of juveniles.

<u>March</u> - Census was made on 20.3.78. The atmospheric temperature was 16.0°C and humidity 50%. 24 frogs (3 males, 1 female and 20 juveniles) were collected in 3 hours 50 minutes. The SVL of the frogs in the sample ranged from 3.00 cm to 5.6 cm. The median SVL being 3.4 cm and showed two modes of 3.4 and 4.0, showing the predominance of juveniles. Males were, however, more in number in this collection than in previous collections.

<u>April</u> - Census was made on 12.4.78. The atmospheric temperature was 21.0°C and humidity 70%. 26 frogs (7 males, 5 females and 14 juveniles) were collected in 3 hours 45 minutes. The SVL of the frogs in the sample ranged from 3.2 cm

Table 3.2

Catch/hour density of frogs at Pologround, Shillong

Date	Temp °C	Humidity %	Number of frogs collected	Duration hr	Duration min	Snout vent length size group 1.0 cm class interval 2.00–2.90	3.00–3.90	4.00–4.90	5.00–5.90	6.00 above	Median	Mode	Catch per hour	Sex ratio M:F:J
5. 1.78	8.0	70	20	5	0	14	4	1	1	-	2.8	2.8	4	1:0:19
24. 2.78	11.5	65	22	4	0	2	15	3	2	-	3.7	3.1 & 3.7	5.5	2:1:19
20. 3.78	16.0	50	24	3	50	-	12	10	2	-	4.0	3.4 & 4.2	6	3:1:20
12. 4.78	21.0	70	26	3	45	-	7	11	5	3	4.5	4.5 & 3.5	7	7:5:14
7. 5.78	18.0	85	50	5	20	-	6	30	12	2	4.5	4.1	9	11:4:10
17. 6.78	21.5	93	25	3	30	2	10	7	5	1	4.0	3.6	7	3:3:19
8. 7.78	20.5	92	22	5	0	-	3	14	5	-	4.2	4.0	4.5	11:2:9
20. 8.78	23.0	75	21	6	0	-	3	10	5	3	4.7	4.6	3.5	7:5:8
16. 9.78	19.5	74	20	3	0	-	4	8	4	4	4.7	4.3	6.5	3:3:4
9.10.78	18.0	84	21	3	20	1	5	9	4	2	4.4	4.2	6.0	7:3:11
18.11.78	13.0	86	22	3	45	2	15	4	1	-	3.7	3.5	6.0	3:1:18
18.12.78	10.2	70	20	4	0	12	7	1	-	-	2.8	2.8	5.0	1:0:19

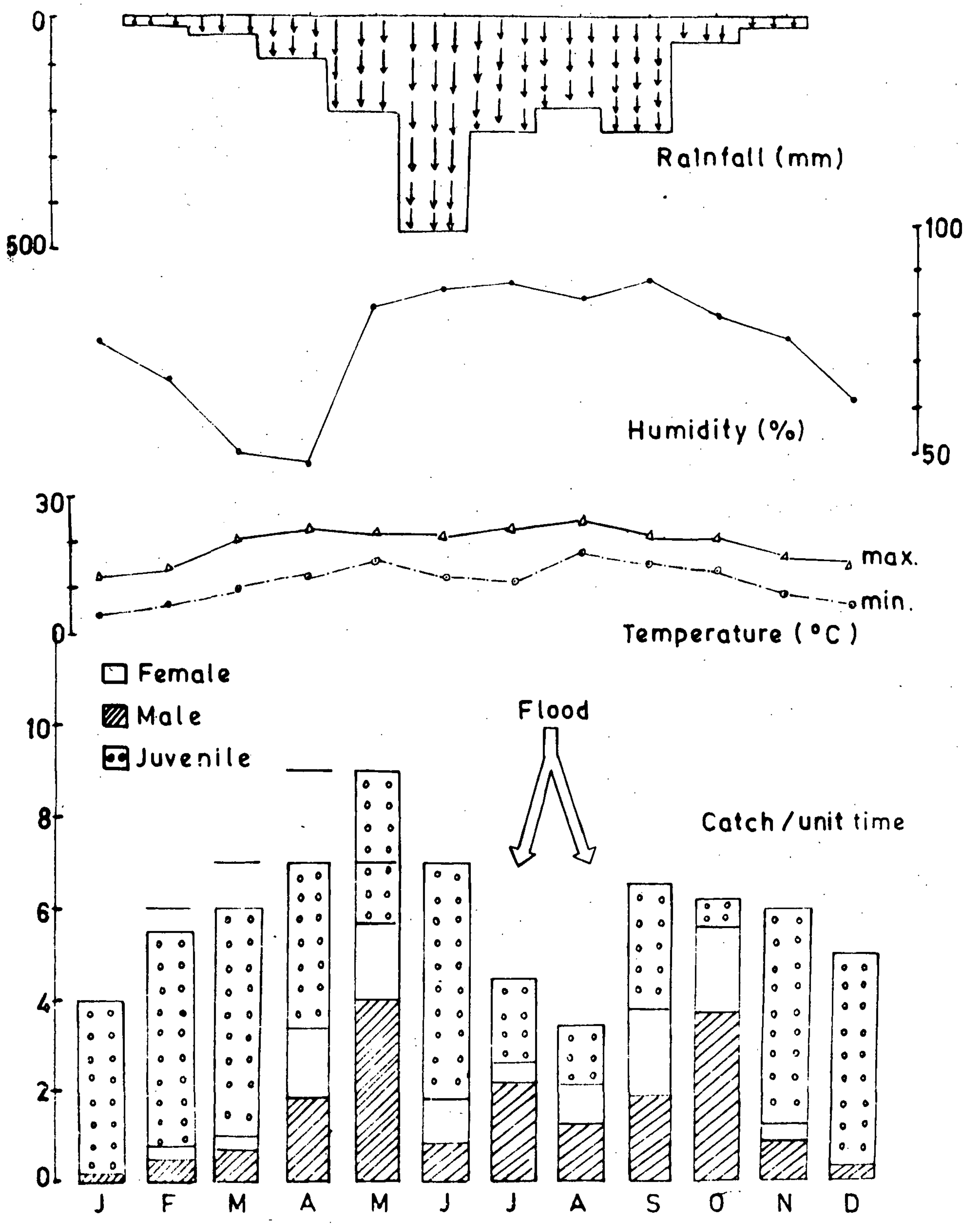
to 6.6 cm. The median SVL being 4.5 cm and showed modes of 3.5 and 4.5 cm, showing marked increase in adult population.

May - Census was made on 7.5.78. The atmospheric temperature was 18.0°C and humidity 85%. 50 frogs (22 males, 8 females and 20 juveniles) were collected in 5 hours 20 minutes. The SVL of the frogs in the sample ranged from 3.3 cm to 6.2 cm. The median SVL being 4.5 cm and showed a mode of 4.1 cm, showing the large abundance of frog on land, predominated by males.

June - Census was made on 17.6.78. The atmospheric temperature was 21.5°C and humidity 93%. 25 frogs (3 males, 3 females and 19 juveniles) were collected in 3 hours 30 minutes. The SVL of the frogs in the sample ranged from 2.5 cm to 6.0 cm. The median SVL being 4.0 cm and showed a mode of 3.6 cm, The Umkhrah river has flooded the site, making the catch difficult and catch/hour poor.

July - Census was made on 8.7.78. The atmospheric temperature was 20.5°C and humidity 92%. 22 frogs (11 males, 2 females and 9 juveniles) were collected in 5 hours. The SVL of the frogs in the sample ranged from 3.7 cm to 5.9 cm. The median SVL being 4.2 cm and showed a mode of 4.0 cm , showing poor catch/hour due to flooding and increased water level at Umkhrah site.

Fig. 3.2 : Availability of _Rana cyanophlyctis_ in relation to environmental conditions during different months of 1978 at Shillong site.



Rainfall (mm)
0
500
Humidity (%)
100
50
30
0
max.
min.
Temperature (°C)
Female
Male
Juvenile
Flood
Catch / unit time
10
8
6
4
2
0
J
F
M
A
M
J
J
A
S
O
N
D

Fig. 3.2

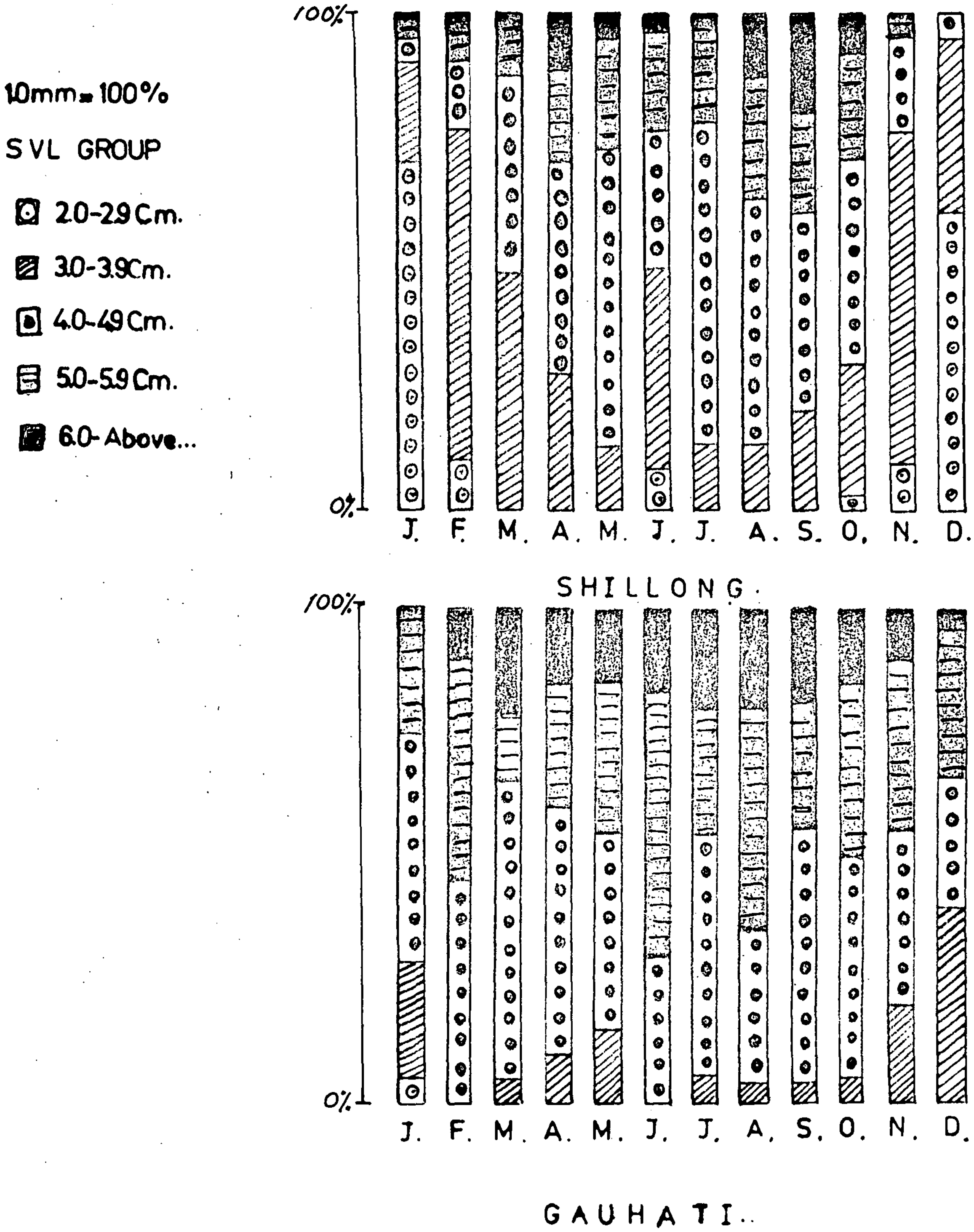
August - Census was made on 20.8.78. The atmospheric temperature was 23.0°C and humidity 75%. 21 frogs (7 males, 6 females and 8 juveniles) were collected in 6 hours. The SVL of the frogs in the sample ranged from 3.5 cm to 6.7 cm. The median SVL being 4.7 cm and showed a mode of 4.6 cm, showing the population on land with almost equal number of male, female and juveniles, however, the catch/hour was very low due to flood at the site.

September - Census was made on 16.9.78. The atmospheric temperature was 19.5°C and humidity 74%. 20 frogs (6 males, 6 females and 8 juveniles) were collected in 3 hours . The SVL of the frogs in the sample ranged from 3.2 cm to 6.8 cm. The median SVL being 4.7 cm and showed a mode of 4.3 cm, showing almost equal numbers of male, female and juvenile.

October - Census was made on 9.10.78. The atmospheric temperature was 18.0°C and humidity 84%. 21 frogs (7 males, 3 females and 11 juveniles) were collected in 3 hours 20 minutes. The SVL of the frogs in the sample ranged from 2.8 cm to 6.2 cm. The median SVL being 4.4 cm and showed a mode of 4.2 cm, The population showed increase in juveniles number and decrease in adult number.

November - Census was made on 28.11.78. The atmospheric temperature was 13.4°C and humidity 86%. 22 frogs (3 males, 1 female and 18 juveniles) were

Fig. 3.13 : Percentage abundance of various size group SVL in a population of <u>Rana cyanophlyctis</u> during different months at Gauhati and Shillong sites.



10mm = 100%
SVL GROUP
2.0-2.9 Cm.
3.0-3.9 Cm.
4.0-4.9 Cm.
5.0-5.9 Cm.
6.0- Above...
100%
0%
J. F. M. A. M. J. J. A. S. O. N. D.
SHILLONG.
100%
0%
J. F. M. A. M. J. J. A. S. O. N. D.
GAUHATI..

Fig. 3.3

collected in 3 hours 45 minutes. The SVL of the frogs in the sample ranged from 2.8 cm to 5.8 cm. The median SVL being 3.7 cm and showed a mode of 3.5 cm, showing the predominance of juveniles.

<u>December</u> - Census was made on 18.12.78. The atmospheric temperature was 10.2°C and humidity 70%. 20 frogs (1 male, 0 female and 19 juveniles) were collected in 4.0 hours. The SVL of the frogs in the sample ranged from 2.4 cm to 4.2 cm. The median SVL being 2.8 cm and showed a mode of 2.8 cm, showing the disappearance of adult specially female in water bodies. Catch predominated by juveniles.

Terrestial availability and relative abundance of various SVL size group at the two sites during different months of 1978 have been estimated. The data has been compiled and presented in Fig. 3.3. During population census it has been observed that predominance of the juveniles and small size frogs have been more than adult and large size frogs during winter months. In summer months and on rain the population of large size frogs increased tremendously. The most active SVL size group at two sites and subsequently most caught were frogs of SVL varying from 4.0 - 4.9 cm. The percentage of various size group in the terrestial population of <u>Rana cyanophlyctis</u> have also been estimated during different month of Gauhati and Shillong site. The data has been compiled and presented in Fig. 3.4. It has been observed that at both the sites, the frogs of SVL varying from 4.0 - 4.9 cm predominated the population census. Further, frogs with SVL 3.0 - 3.9 cm and 5.0 - 5.9 cm also showed higher percentage of occurrence at Shillong and Gauhati site respectively.

(b) <u>Capture, mark release and recapture technique</u>

(Site: State Government Fish Farm Ulubari, Gauhati, Assam)

The principle of the capture, marked release and recapture technique is based on the analysis of re-occurrance of a known (marked) population within an unknown population inhabiting the same area (Jackson, 1936, Jameson 1957). The recapture analysis of the known members with unknown members reveals the abundance of the total population of the species inhabiting a particular site. Taking into consideration the limitations of the technical difficulties in the the methodology it is presumed that:

1. Marked animals, once released in the study site become distributed homogeneously with the unmarked individuals of the population. There after the chances of recapture stand as good as the chance of capture of unmarked individuals in that populations (50% probability).

2. To minimize the bias, the frogs in each catch were collected at random, throughout the site area.

3. The marked frogs would neither die nor cross out the study site area and the total population density of the species would remain unaffected as, supposedly there is no migration or immigration

4. In case of any migration it is believed that equal number might have come in from neighbourhood.

Initially, marking of the frogs with the help of toe clipping, paints and polish have been attempted, but it did not prove successful. Later, marking by banding coloured thread around thighs were used with success and subsequently during every census, the frogs were marked and released at a middle point of the study-site. After a lapse of 24 hr (a period allowed for homogenous mixing of the marked and released frogs, with the unknown, unmarked population of the frog at the study site) the frogs were collected at random, covering the whole area of the study site. The frogs thus collected contained marked as well as unmarked individuals. The number of marked and released frogs, captured marked and unmarked frogs was deduced in Jameson (1957) formula (see material and method) to find out the numbers of unmarked individuals in the population occupying the study area. The estimated number of unmarked frogs with the total number of marked and released frog was combined to estimate the abundance of frogs in that area. Since all collection, were restricted to land and not to the water bodies, the present estimation gives us an idea of the relative abundance of the frog population on the land only.

The investigation at Gauhati were carried out from January to December 1978. A minimum of 4 estimations of the relative abundance of frog, in a month, were made. The average of the 4 such estimates, represented the average abundance of the frog on the land during that particular month. In September and December 5 estimates of the relative abundance were made. Thus, during 12 months of investigation altogether 50 estimates were made. The data has been compiled and presented in Table 3.3. Of the relative density, on precensus days

(Jameson, 1957: the day of marking and release) frogs were collected for 2.0 hours marked and released. On census day (Jameson, 1957: second day for recapture analysis) frogs were collected at randomly (as described in technique) and the number of marked frogs recaptured and unmarked frog capture were known. The numbers of marked and unmarked frogs were subjected to Jackson's formulae and subsequently the relative abundance was calculated. The data has been compiled in Table 3.3. The month-wise observations are described below:

<u>January</u> - During investigation period the atmospheric temperature ranged between 9.3°C to 22.8°C, and there was no rainfall. At precensus 11, 19, 25 and 32 frogs were marked and released. The number of frogs recaptured on the subsequent days were 2/10, 4/10, 8/15 and 9/16 (numerator represents the marked catch and demoninator total number caught). The ratio between the total marked and unmarked frogs collected during census were approximately 1:1. The estimated relative abundance of the frog on land varied between 46 to 47 ($\bar{X}$: 53).

<u>February</u> - During investigation atmospheric temperature ranged between 10.6 to 26.5°C and total monthly rainfall was 3.6 mm. At precensus 15, 31, 44 and 47 frogs

were marked and released. The number of frogs recaptured on the subsequent days were 2/18, 5/18, 7/20 and 9/22. The ratio between the total marked and unmarked frogs collected during census was approximately 1:2. The estimated relative abundance of the frog on land varied between 104 to 135 ($\bar{X}$: 120).

<u>March</u> - During investigation atmospheric temperature ranged between 14.8°C to 31.0°C and total monthly rainfall was 15.1 mm. At precensus 24, 41, 70 and 80 frogs were marked and released. The number of frogs recaptured on the subsequent days were 5/30, 7/28, 10/30 and 15/34. The ratio between the total marked and unmarked frogs collected during census was appoximately 1: 25. The estimated relative abundance of the frog on land varied between 144 to 210 ($\bar{X}$; 189) . At the warm night of 9.3.1978 the population estimated were maximum.

<u>April</u> - During investigation atmospheric temperature ranged between 19.1°C and 31.0°C and total monthly rainfall was 62.4 mm. At precensus 24, 56, 70 and 80 frogs were marked and released. The number of frogs recaptured on the subsequent days were 4/34, 8/22, 9/25 and 14/38. The ratio between the total marked and unmarked frogs collected during census was approximately 1:25. The estimated relative

Table 3.6

Estimation of relative abundance in <u>Rana cyanophlyctis</u> by capture, marked released and recapture method (Jackson 1936) at Ulubari Gauhati

Date of experiment	Atm. Temp °C	Total Rain-fall mm	Frogs marked and released	Number captured during census	Duration of collection hr	min	Marked captured	Unmarked captured	Un-marked population	Total population	Range	Mean
10. 1.78	9.3		11	10	2	20	2	8	44	55	46	
11. 1.78	to	't'	19	10	2	30	4	6	27	46	to	53
12. 1.78	22.8		25	15	3	00	8	7	28	53	57	
13. 1.78			32	16	3	00	9	7	25	57		
14. 2.78	10.6		15	18	3	00	2	16	120	135	104	
15. 2.78	to	3.6	31	18	3	00	5	13	72	104	to	120
16. 2.78	26.5		44	20	3	00	7	13	72	116	135	
17. 2.78			47	22	3	20	9	12	69	125		
7. 3.78	14.8		24	30	3	00	5	25	120	144	144	
8. 3.78	to	15.1	41	28	3	00	7	21	147	196	to	189
9. 3.78	31.0		70	30	3	00	10	20	140	210	210	
10. 3.78			80	34	3	00	15	19	114	204		
4. 4.78	19.1		24	34	2	40	4	30	195	229	154	
5. 4.78	to	62.4	56	22	2	00	8	14	98	154	to	201
6. 4.78	31.0		70	25	2	20	9	16	124	194	233	
7. 4.78			86	38	3	15	14	24	147	233		
6. 5.78	22.3		28	36	3	00	3	30	280	308	156	
7. 5.78	to	243.8	58	35	3	00	13	22	98	156	to	221
8. 5.78	31.2		80	31	3	00	14	17	97	117	308	
9. 5.78			97	50	3	30	20	30	146	243		
8. 6.78	24.2		24	25	2	00	3	21	168	192	124	
9. 6.78	to	364.8	45	30	2	30	11	19	79	124	to	167
10. 6.78	31.7		64	32	2	30	14	18	81	145	205	
11. 6.78			82	45	3	20	18	27	123	205		
2. 7.78	24.7		15	16	1	00	1	15	225	240	177	
3. 7.78	to	308.8	23	14	1	00	2	12	180	193	to	226
4. 7.78	31.9		42	42	3	10	10	32	135	177	296	
5. 7.78			74	48	3	00	12	36	222	296		
8. 8.78	24.1		24	28	2	20	3	25	200	227	227	
9. 8.78	to	237.7	49	26	2	00	4	22	269	318	to	291
10. 8.78	33.09		71	32	2	30	8	24	213	284	337	
11. 8.78			95	39	2	50	11	28	242	337		
14. 9.78	42.2		30	24	2	30	4	20	150	174	125	
15. 9.78	to		50	10	1	20	4	6	75	125	to	
16. 9.78	32.30	270.5	56	22	2	30	8	14	98	154	180	160
17. 9.78			70	18	2	00	7	11	110	180		
18. 9.78			81	20	2	00	10	10	81	162		
9.10.78	21.8		20	18	2	10	3	15	100	120	103	
10.10.78	to	33.2	35	17	2	00	5	12	84	119	to	119
11.10.78	31.45		47	23	2	30	9	14	56	103	134	
12.10.78			61	22	2	30	10	12	73	134	134	
26.11.78	16.73		15	17	2	20	3	14	70	85	69	
27.11.78	to	42.7	19	19	2	30	7	12	50	69	to	91
28.11.78	26.27		41	25	3	00	11	14	53	94	114	
29.11.78			45	27	3	00	13	14	59	114		
9.12.78	10.23		5	13	2	00	1	12	60	65	65	
10.12.78	to		17	14	2	00	2	12	102	119	to	
11.12.78	23.27	't'	19	17	2	00	5	12	69	88	119	89
12.12.78			41	13	2	00	6	7	48	89		
13.12.78			50	12	2	00	7	5	34	82		

abundance of the frog on land varied between 154 to 233 ($\bar{X}$: 201).

<u>May</u> - During investigation atmospheric temperature ranged between 22.3°C and 31.2°C and total monthly rainfall was 243.8 mm. At precensus 28, 58, 80 and 97 frogs were marked and released. The number of frogs recaptured on the subsequent days were 3/36, 13/35, 14/31 and 20/50. The ratio between the total marked and unmarked frogs collected during census was approximately 1:2. The estimated relative abundance of the frogs on land varied between 156 to 308 ($\bar{X}$; 221). The relative abundance was maximum soon after rains (243 and 308).

<u>June</u> - During investigation atmospheric temperature ranged between 24.2°C and 31.7°C and total monthly rainfall recorded were 364.8 mm. At precensus 24, 45, 64 and 82 frogs were marked and released. The number of frogs recaptured on the subsequent days were 3/25, 11/30, 14/32 and 18/45. The ratio between the total marked and unmarked frogs collected during census approximately 1:2. The estimated relative abundance of the frog on land varied between 124 to 205 ($\bar{X}$: 167).

July - During investigation atmospheric temperature ranged between 24.7°C and 31.9°C and total monthly rainfall recorded were 308.8 mm. At precensus 15, 23, 42 and 74 frogs were marked and released. The number of frogs recaptured on the subsequent days were 1/16, 2/14, 10/42 and 12/48. The ratio between the total marked and unmarked frogs collected during census was approximately 1:3.5. The estimated relative abundance of the frog on land varied between 177 to 296 ($\bar{X}$: 226).

August - During investigation atmospheric temperature ranged between 24.1°C to 33.09°C and total monthly rainfall recorded were 237.7 mm. At precensus 24, 49, 71 and 95 frogs were marked and released. The number of frogs recaptured on the subsequent days were 3/28, 4/26, 8/32 and 11/39. The ratio between the total marked and unmarked frogs collected during census was approximately 1:4. The estimated relative abundance of the frog on land varied between 224 to 337 ($\bar{X}$: 291)

September - During investigation atmospheric temperature ranged between 24.2°C and 32.30°C and total monthly rainfall was 270.5. At precensus 30, 50, 56, 70 and 81 frogs were marked and released. The number of frogs recaptured on the subsequent days were 4/24, 4/10, 8/22, 7/18 and 10/20. The ratio between the total marked and unmarked frogs collected during census was approximately 1:2. The estimated relative abundance of the frog on land varied between 125 to 180 ($\bar{X}$: 160).

<u>October</u> - During investigation atmospheric temperature ranged between 21.8°C and 31.45°C and total rainfall recorded were 33.2 mm. The relative abundance of the frogs decreased further. At precensus 20, 35, 47 and 61 frogs were marked and released. The number of frogs recaptured on the subsequent days were 3/18, 5/17, 9/23 and 10/22. The ratio between the total marked and unmarked frogs collected during census was approximately 1:2. The estimated relative abundance of the frog on land varied between 103 to 134 ($\bar{X}$: 119).

<u>November</u> - During investigation atmospheric temperature ranged between 16.73°C and 26.27°C and total monthly rainfall recorded were 42.7 mm. At precensus 15, 19, 41 and 45 frogs were marked and released. The number of frogs recaptured on the subsequent days were 3/17, 7/19, 11/25 and 13/27. The ratio between total marked and unmarked frogs collected during census was approximately 1:1.5. The estimated relative abundance of the frog on land varied between 69 to 114 ($\bar{X}$: 91).

Fig. 3.9 : Availability of various size group (SVL) in *Rana cyanophlyctis* population during different months at Gauhati and Shillong.

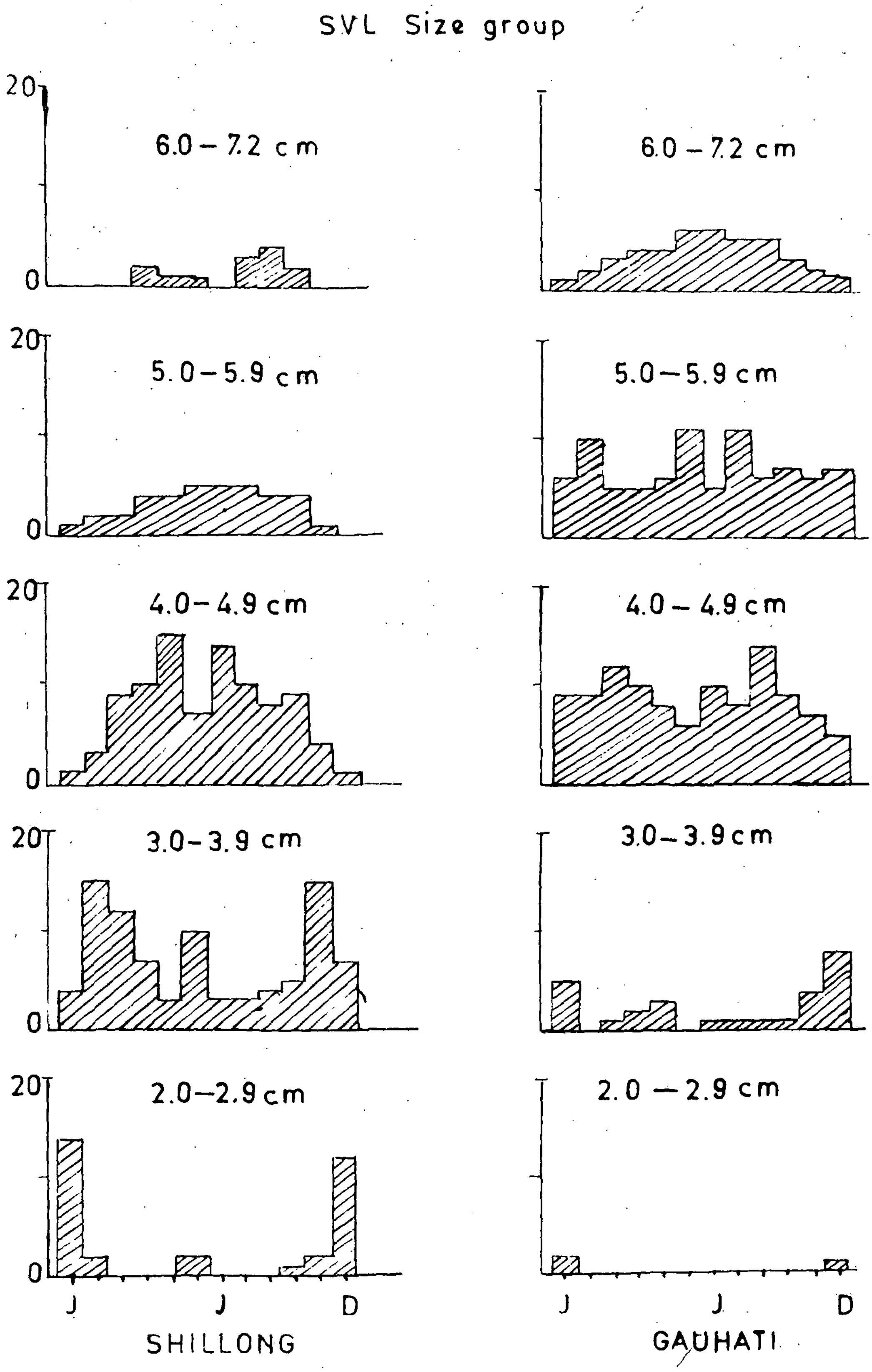

SVL Size group
20
0
6.0 – 7.2 cm
5.0 – 5.9 cm
4.0 – 4.9 cm
3.0 – 3.9 cm
2.0 – 2.9 cm
J
J
D
SHILLONG
GAUHATI

Fig. 3.9

December - During investigation atmospheric temperature ranged between 10.23°C and 23.27°C and total monthly rainfall was nil. At precensus 5, 17, 19, 41, 48 frogs were marked and released. The number of frogs recaptured on the subsequent days were 1/13, 2/14, 5/17, 6/13 and 7/12 . The ratio between the total marked and unmarked frogs collected, during census was approximately 1:2. The estimated relative abundance of the frog on land varied between 65 to 119 ($\bar{X}$: 89).

It has been observed that the relative abundance of the frog showed an increasing trend from January till August and later a downward trend, till it reaches to a minimum during December and January. It was estimated that during January the relative abundance were minimum 46 - 57 (X = 53) and during August it was maximum 224 - 337 (X = 291). It has also been noted that relative abundance of frogs on land increased enormously during monsoons.

The changes in the relative density of the frogs can be correlated with temperature, humidity and rainfall.

Temperature:

The atmospheric and aquatic temperatures are in-interdependent on each other (Table 1.1.1) and correlation coefficient between the two variable was found to be highly significant ($P < 0.01$; $r = 0.85$).

The relative density of frogs on land was lowest (53) in January and maximum (271) in August. This trend of fluctuation is similar as that of atmospheric temperature (January 9.3°C ; August 24.1°C ; December 10.23).

The correlation between the two variables density and temperature has been represented by linear relationship (Table 3.4 ; Fig. 3.5) the bivariate correlation coefficient showed high coefficient constant ($r = 0.83$) and was found significant on 'F' test ($P < 0.01$).

<u>Humidity</u>:

The humidity at the study site fluctuated between 69% (minimum) during March and 91% (maximum) during June, with an annual average of 82.5%. The high humidity has been attributed Brahmaputra and Bharlu rivers (Karunkaran 1974) at the close vicinity of the study site. The relative abundance (Table 3.4) and humidity at the study site do not show any correlation. There was also no significant correlation between the humidity and temperature ($r = 0.31$) and also between humidity and rainfall ($r = 0.41$).

Fig. 3.6 : Correlation of relative abundance of *Rana cyanophlyctis* with temperature

3.7 : Correlation of relative abundance of *Rana cyanophlyctis* with pH

3.8 : Correlation of relative abundance of *Rana cyanophlyctis* with rainfall.

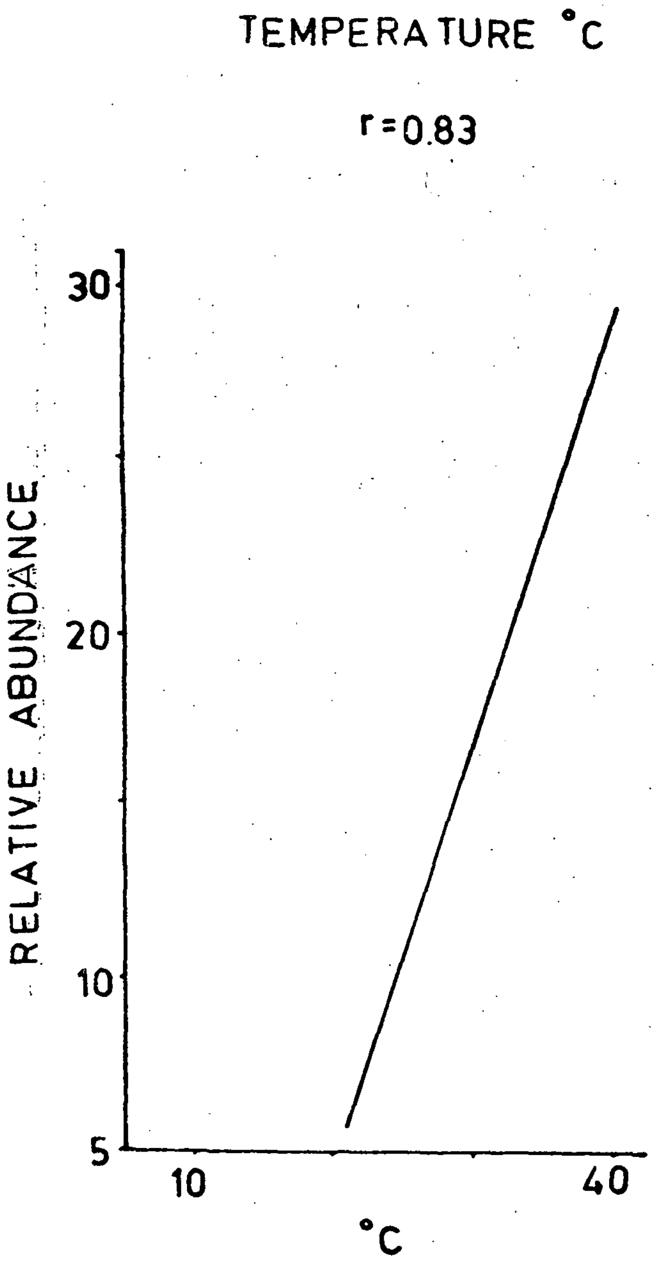


Fig. 3·6

Fig. 3·7

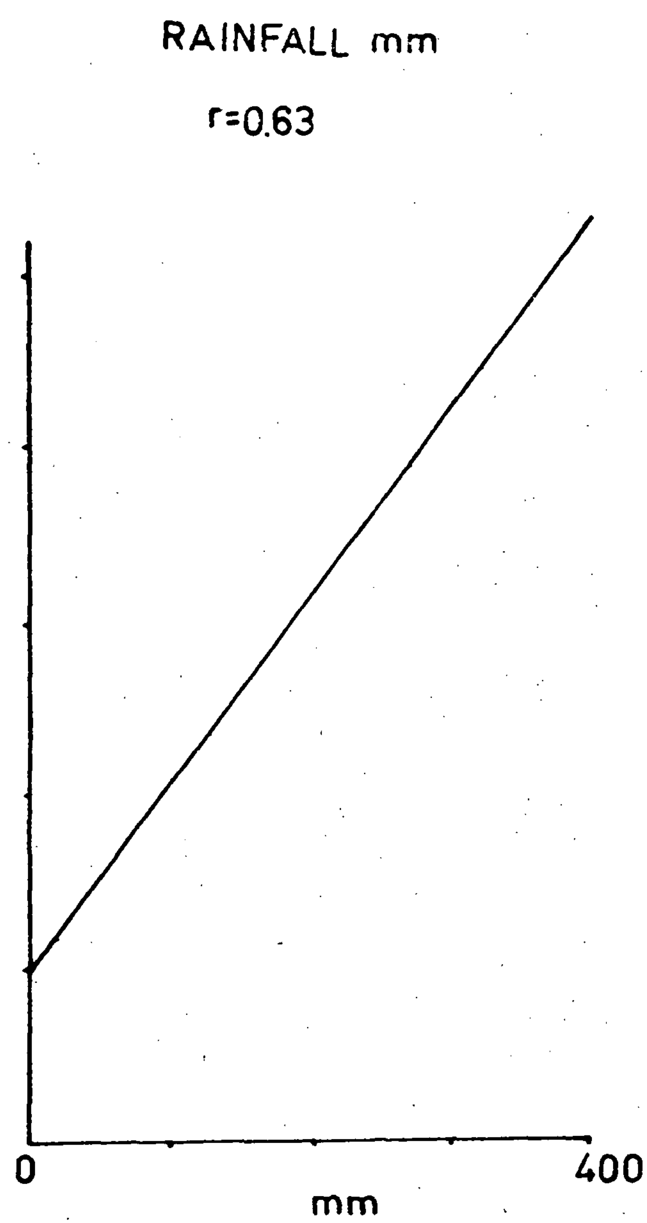


Fig. 3·8

Rainfall:

At Gauhati, the maximum precipitations (243.8 mm, 364.8 mm and 270.5 mm) occurred from May to September when the atmospheric temperature was also recorded to be maximum. The correlation coefficient derived between the temperature and rainfall has been found significant on 'F' test ($r = 0.84$, $P < 0.01$). The trend in the changes in the rainfall has been found similar with the relative abundance of the frog populations . The relationship shows a linear expression (Table 3.4 ; Fig. 3.7). The correlation coefficient derived between the two variables have also found to be significant on 'F' test ($r = 0.63$: $P < 0.05$).

Chemical factors:

Chemical factors analysed have been shown in Figs. 1.1.5 and 1.2.4.

pH - Throughout the period of investigation the pH of the site water was found alkaline. The maximum pH 8.3 was recorded during January and was found decreasing till it reached almost neutral (7.0) during rainy season, and then it further increased till it reached maximum 7.9 during December (Fig. 1.1.5). Rain and pH have been found to be inversely related. The correlation coefficient calculated between the rainfall and pH and rainfall and temperature have found to be significant ($r = 0.81$ and $r = 0.65$; $P < 0.05$). Maximum relative abundance of the frogs was observed during the months when pH was neutral (7.0). The correlation coefficient between the two variables have also been found significant ($r = 0.85$; $P < 0.001$) on 'F' test.

During the period of every population census, conductivity, chloride bicarbonate, calcium, magnesium and total hardness of the pond water have also been analysed. The annual fluctuations of these factors showed no definite trend (Fig. 1.1.2). The periodical manuring of the ponds by the fish department results in the sudden hike in the dissolved ionic content, including the conductivity of the water bodies. This accounts for high conductivity (378.0 micromhos/mm), hardness (150 ppm) and other ionic presence. The fluctuation of chemical factors in January, August and December was : conductivity 181.0, 335.0 and 316.80 micromhos/mm ; chloride 3.20, 44.0 and 28.0 ppm ; bicarbonate 70.0, 162.0 and 171.0 ppm ; calcium 11.0 , 28.0 and 25.0 ppm ; magnesium 5.50, 13.00 and 19.00 ppm ; total hardness 50.0, 125.0 and 140.0 ppm respectively. The correlation coefficient between various bivariate

Table 3.4

Values of correlation coefficient derived between terrestial availability of <u>Rana cyanophlyctis</u> and environmental condition at Gauhati & Shillong study site

Juvenile population	Female population	Male population	Catch/ hour	Variables	Catch/ hour	Male population	Female population	Juvenile population
0.7332**	0.6056*	0.7210**	0.7855**	Temperature	0.2318	0.7743**	0.8524**	0.8119**
0.3238	0.0407	0.1329	0.0177	Humidity	0.2349	0.3898	0.6295*	0.5519
0.5143	0.4665	0.5800*	0.7977**	Rainfall	0.2236	0.4125	0.5461	0.4045
0.8900**	0.6719*	0.6775*	0.7211**	pH	0.2982	0.7048*	0.7673**	0.6309*
0.1533	0.6276*	0.0460	0.3665	Conductivity	0.1336	0.2238	0.1263	0.0088
0.0924	0.6140*	0.1280	0.2988	Total Hardness	0.3528	0.0263	0.2585	0.0156
0.8119	0.6001	0.6024	0.7388	Multiple Correlation	0.6077	0.6893	0.7162	0.7888

* Significant at 5% probability level

** Significant at 1% probability level

limnological combinations conductivity, bicarbonate, chloride, calcium, magnesium and total hardness have been found to be significant ($P < 0.01$) on 'F' test (Table 1.1.2). These factors may be helpful for the growth and maintenance of larval population and indirectly to the frog population. Direct relationship of these with adult frogs cannot be established.

3.4.3. Home Range and movement:

The investigation on frog abundance also reveals that Rana cyanophlyctis maintains a home range. Following account deals with home range and movement during 3 phases of annual breeding cycles of this frog.

Pre-breeding phase:

Following an analysis on home range movement of this frog at Gauhati, in addition to abundance of frogs by catch per hour the analysis of relative abundance by capture recapture method and the distance up to which frogs moved away from the release point was recorded. During prebreeding phase in February, March, April and first fortnight of May. The average atmospheric temperature ranged from 7.3°C to 22.6°C and rainfall 3.6 mm to 62.4 mm.

In winter months due to the lowering of water level in most of the nursery ponds, the frogs lived in aggregation in a breeding pond. During February they are seen dispersing. The random capture sample of frogs on land in February represented a ratio of 8 juveniles, 3 males and 1 female indicating the predominance of juvenile frogs. This indicates movement of the juveniles frogs was more than the adults one. With the increase in atmospheric temperature during March the frog particularly males become more active as out of 41 frogs collected in March there were 16 juveniles, 17 males and 8 females. The atmospheric temperature further increased to appeaxmately 26.6°C in April and May and the rainfall was over 60.0 mm. These factors seem to be favourable as increased number of frogs were found moving on land. In May, out of 51 frogs collected the ratio was 19 juveniles, 17 males and 15 females. The minimum distance travelled by any frog from its release point was 19 m, during February, and maximum 34.0 m during May. So far as size is concerned, predominantly smaller size frogs (SVL 4.0 - 5.6 cm) were frequently observed in February and large size ones (3.5 - 6.4 cm) in April. This indicates that frogs of allsize and and sex become active by this time and start moving on land.

Breeding phase:

During middle of May, June, July and August temperature was 22.3°C - 31.9°C and rainfall was 243.8 - 364.8 mm. The population of *Rana cyanophlyctis* was most active during this period. The SVL of the frogs collected during this period ranged from 3.5 cm to 6.7 cm. The accessibility and collection of the frogs was also fairly high, as 11 to 14 frogs were collected per hour. The movement range of the frogs was much larger as marked frogs were captured as far as 128 m from the release point. 2 marked frogs were observed to have crossed the study site territory and moving in the eastward low lying area. A relative density census m made at warm night (1.5.78), soon after heavy downpour showed exceptionally high capture, as 50 frogs were captured in 2 hours 20 minutes.

Post-breeding phase:

During September, October, November, December and January, the atmospheric temperature varied from 9.3°C to 32.0°C and rainfall decreased from 270.5 mm to 0.0 mm. A decline in the movement and activities of the frogs was noted during this period. Their movements was gradually restricted near the vicinity of water bodies. A higher

Fig. 3.4 : Basking of frogs during winter

3.5 : A view of home site and movement in the population of Rana cyanophlyctis.

Fig. 3·4

Fig. 3·5

rainfall was usually recorded on warm evening inducing increased movement and activities of frog population. During winter rains the activities of frog population remain restricted at close vicinity of water bodies. During August and September the ratio of males and females and juveniles was almost equal as out of 46 frogs collected 16 were males, 15 were females and 15 juveniles. However, in November the predominance of juveniles was increased. The adults were observed to remain restricted in water bodies. The SV length of the frogs collected varied from 3.7 cm to 6.6 cm. During winter large size adults occupied central deep portion of the water bodies whereas the sub-adult and immature frogs peripheral shallow portion of the water bodies close to the eldges. The frogs do not inhabit those ponds which dry up during this period. Whenever there was slight rain or warm weather, the movement of juveniles and adult males were observed. Female rarely moved out. The maximum distance travelled by a marked frog from its release point during winter was 14.0 m. The frogs sitting on edges and basking are often observed during winter months (Fig. 3.4). On approach or disturbance their quick disappearance, in water bodies are noticed.

DISCUSSION

Marking of anurans

The toe clipping technique for marking anurans described by Martof (1953) was successfully used by Dole (1965), Dole and Durant (1965, 1974), however, in the present investigations it did not prove helpful. It did not cause death to the animal but certainly affected the skipping and free swimming. The frogs with clipped toes which includes a portion of welb also, preferred to remain buried in the mud. Clarke (1974) also recorded that toads with clipped toes remain less active and showed reduced movement. He preferred injection of radio active isotopes, manganese-52 at the dorsal side beneath skin and observed that such frogs could be detected in the field by Thyae III survey meter, equipped with a 3.18 x 3.18 Na-71 crystal gamma scintillation probe. It was not possible to try such technique here due to non-availability of equipment and isotopes. The techniques preferred by Andrewartha (1961) and Krekorian (1976) to mark the animal with paint and nail polish did not work with Rana cyanophlyctis as it fades out very fast. The use of different colour thread as bends followed by Lincoln (1930) and Andrewartha (1961) around thigh of the animal, proved very convenient and successful and hence was followed in the present investigation. The other technique of marking anurans with the help of copper wires and iron rings (Richter 1973; Dougherty 1976 and Krekorian 1976) was not attempted.

Relative abundance:

Although various methods have been described for the estimation of relative density of a populations among insect and invertebrate (see Lincoln 1930; Gause 1932; Jackson 1936,1939,1940,1944,1948; William 1940; Johnson 1950,1952,1954; Banks 1954; Browning 1959,1963; Andrewartha 1957,1961) very few techniques are available for vertebrates.

The capture, marked released and recapture method profounded by Jackson (1936) have been successfully used for various animal species, such as, duck (Lincoln 1930), Tse-tse fly (Jackson 1936,1939,1940,1944) and 1948), Polyommatus iracus (Dowdeswell et al. 1940; Dowdeswell 1952), Mus musculus (Evans 1949) in field rats (Leslic and Davis 1939), Dacus cucurbitae (Ito et al. 1974), fishes (Groeking 1952); Watervoles rodents (Worral 1964, Zejda 1972; Holisova and Pelikan 1974; Airoldi 1977), hylid frogs (Jameson 1957) and in buds (Manly 1977). In the present investigation, it has been used to know relative density and abundance of Rana cyanophlyctis population available on land, adjoining ponds at the study site. The frogs were collected at random in a homogenously mixed population of the marked individuals released earlier and unmarked, individual present in the habitat. The ratio of the animals, marked and released earlier (known population) and recaptured with unmarked (the population dynamics in the study site) reveals a trend enabling for the calculation of relative density of the frog..

The relative density during different months has also been estimated by analysing the number of catch per unit time. Lowest number of frog were available in January. The number went on increasing till August and then there was decline in their availability. This method for the estimation of relative density has been followed by earlier worker such as Currie and Bellis (1969) for *Rana catesbeiana* , Zejada (1972) for water voles; Hazlett *et al.* (1974) for Cray fish *Orconectis virilis*; Koskela and Pasanen (1974,1975) for *Rana temporaria*, Roy (1979) for *Rana limnocharis*.

Both the techniques (1) Jackons's method, (2). Number catches per unit time, revealed similar trend. The poor density of frogs during winter months when temperature and rainfall were minimum and high density during summer months when rainfall was also high was recorded.

Home range, movement and environmental factors:

Home range and movements

The area of daily activity and movement of animals around the home site has been defined as home range (Bert 1943; Carpenter 1952). Dole and Durant (1965) considers home range as an area where animal return again and again after its daily or routined activity. *Rana cyanophylotis* in the present investigation return again and again to same place and were recaptured clearly justifies that the frog has a home range. The 'home range' or 'activity range' have been observed in various tropical and sub-tropical anurans (See Martof 1953; Turner 1960; Bellis

1965; Dole 1965; Jameson 1955,1957; Carpenter 1954; Sexton 1960; Fitch 1956 and Pearson 1955) and have been reported to vary, subject to dependence on food (Kilby 1945); body size (Carpenter 1952); Shelter sites (Jameson 1956, 1957) or on various factor together, such as shelter, food and environmental conditions (Grobman 1950; Dole 1965). Since, study stie Gauhati is well managed by the fisheries department none of theme factors may have any role in home range movement of frogs and is found to be related mostly with the environmental condition. This is further supported by high correlation coefficient between abundance, movement and environmental condition of the site (Table 3.4).

During cold months the frogs were observed restricted to the water bodies, female and large size frogs occupying the deep central core of the water bodies and male and small size the shallow edges. Rana clamitans (Martof 1953, 1956) have also been reported behaving in the similar way during winter months. Rana cyanophlyctis often basks during winter at Gauhati and Shillong sites. Martof (1953) in Rana clamitans, Hansen (1955) in Rana heckscherii Hadfield (1966) in Bufo woodhousi and Fitch (1956) in Gastrophryne olivaceae have observed ectodermal basking and found that it enhances body temperature, metabolic activities and digestion in anurans. The basking in Rana *cyanophlyctis* may also be attributed to the above factors.

On the termination of winter and with gradual increase in atmospheric temperature these frogs showed increased movement from the released site or water bodies. Maximum movement or home range have been observed during summer months specially during rains. McCann (1933) has also recorded that during monsoon months *Rana cyanophlyctis* become more active and can be found almost anywhere. There appears to be no doubt that this species undertaken long journey during night or else it would be difficult to account for its sudden appearance in the water holes which has been recently excavated. In the present investigations first the male frogs were found to enhance their activity zone (home range) and then during summer especially after rains the female frog extend their home range. The spade foot toad (Jameson 1956), chorus frog (Jameson 1957) and *Rana temporaria* (Koskela and Pasanen 1974) were recorded to behaving in the similar pattern as *Rana cyanophlyctis*. The 'home range' and movement, during present investigation had no relations with body size or sex of the animal. This has also been reported in water vole (Zejdi 1971) *Rana pipiens* (Dole 1965), *Atelopus oxyrhynchus* (Dole and Durant 1974) and *Bufo woodhousei* (Clarke 1974).

Temperature:

During the present investigation at Gauhati and Shillong, it was noted that Rana cyanophlyctis does not hibernate. This appears to be characteristic of aquatic frog (Wells 1977). They were active and moving on the land at a temperature ranging from 2°C to 36°C. Though critical minimum and critical maximum (Stuart 1951) has not been worked out in Rana cyanophlyctis. This range shows high range of temperature tolerance. Boulenger (1920) has recorded the animal from hottest area of Rajasthan when temperature often shoot up above 45°C and also from Srinagar, Quatta and Kullu Valley, floating, skitting and moving over water bodies having icy and frozen edges, confirming the high range of thermal tolerance. The movements and activities of the frogs were observed to be directly related with changing atmospheric temperature. The maximum movement and abundance in all the size group and population was observed during June, July, August and September when atmospheric temperature and rainfall were high (Figs. 1.1.2 and 1.2.2). This movement on land may be related to high moisture content as being a water frog it may not stay away from water for long period. However, time takes for dessication in such conditions have not been worked out. During winter months with minimum rainfall they are seen restricted, near water bodies. Ashby (1969) has made similar observation in the self maintaining population of Rana temporaria. Increased activity during high atmospheric temperature has also been observed by Mullally (1952) in Bufo boreas. Commenting upon enhanced activities of anuran during summer months Martof (1952) Willis et al. (1956), Tait (1969) and Clarke (1974) mentioned that high air temperature increases the substrate temperature and subsequently the animal temperature by radiation, which govern the activities in anurans. In Rana cyanophlyctis enhance activities with increased atmospheric temperature and rainfall were found significantly correlated at both the sites The observation of Bohnsack's (1957) in Rana temporaria and Dole and Durant's (1974) in Antilopes that the temperature has little or no correlation with the abundance and movement of the above described frogs, could not be substantiated in Rana cyanophlyctis.

During winter large size Rana cyanophlyctis were observed occupying the central deep portion of the water and juveniles the shallow edges as also recorded by earlier worker Martof (1953) in Rana clamitans, Currie and Bellis (1969,1974) in Rana catesbeiana.

Humidity and rainfall:

McCann (1933) observed that anurans are basically nocturnal but becomes diurnal and nocturnal both during rainy season and in wet localities. Koskela (1973) recorded increased activities and availability of *Rana temporaria* on rainy day then any other day ($\bar{X}$ = 225; P 0.001). Blair (1953) and Jameson (1957) termination of aggregation, hibernation and increased movement of *Bufo vallicaps* and Pacific tree frog respectively, soon after rains. Metter (1964) described that rain increases the quantity of food types in ambhibious environment, that accelerate the activity and movement of the frog, Dole (1964) consider soil moisture as an important factor for the movement of *Antelops*, than the temperature and food. Hayer (1973) noted that breeding activities in the tropical anurans are governed by numerous of which rainfall constitute an important conditions. Hayer and Bellis (1973) have also recorded significance correlation in heavy rains with mating call, mating success and larval success in leptodactylid frogs. In the present investigation on *Rana cyanophlyctis* an increased activity of the frog with increasing rainfall and humidity all around the year has been observed. The maximum activities have been noted soon after summer rains. Similar observations have been made in *Rana clamitans* (Martof 1953), *Rana temporaria* (Ashby 1969), scaphiopus holbrooki (Rubin 196) and *Rana catesbeiana* (Currie and Bellis 1974). Moreover significant correlation ($P<0.01$) have also been observed between relative abundance of various size and sex of *Rana cyanophlyctis* and rainfall.

Metter (1964) and Koskela (1973) mentioned that relative humidity at and above 84% is an important factor in the movement and activities of *Rana temporaria*, Amorono and Marshall (1960) recorded that combinations of rainfall and temperature as predominant external factor in movement and breeding success of anurans. In the present investigation it is believed that rainfall and temperature jointly affect the movement and activity of *Rana cyanophlyctis* . The correlation with temperature has also been observed to significantly high ($P<0.01$) idicating that the two factors acts jointly at two sites (Table 1.1.1).

Limnological conditions:

In the present investigation limnological condition of the pond water at Gauhati and Umkhera river water at Shillong showed little correlation ($P<0.05$) with the behaviour and activity of the frog. It has been observed that Shillong site had acidic pH and Gauhati site alkaline pH during most part of the year, and it become neutral during rainy month (See Figs. 1.1.3 and 1.2.3). During this neutral phase, maximum activity and abundance in Rana cyanophlyctis population has been observed. Bragg (1950) in spade foot toad; Fingal and Kaplan (1963) in Rana catesbeiana; Cooke (1975) in Bufo bufo and Rana temporaria noted varying range of tolerance in anurans with limnological conditions and observed that maximum activities in the above frog populations are observed during rainy season at pH around 7.0 . In Rana cyanophlyctis the significant correlation coefficient ($P < 0.01$) between pH and relative abundance of the frog population indicates that the pH along with other environmental factors plays an effective role in abundance of frog population.

SUMMARY

This chapter deals with relative abundance and home range movement of Rana cyanophlyctis, and relationship of these with environmental conditions. From November till February (winter months) when temperature is low, the populations of Rana cyanophlyctis remain restricted to water bodies, where adults and large size juveniles occupy the middle core and small juveniles, the shallow water areas on embankment. The movement of frogs during this period is restricted to the water bodies, except juveniles which are often observed moving around their edges also, maintaining a smaller 'home range'. During pre-breeding phase, with an increase in atmospheric temperature (18°C - 28°C), the males and juveniles become active and their relative abundance on land increases. During breeding phase, at an atmospheric temperature 25°C - 35°C, with the onset of rains females also come on the land. At this stage relative abundance of males, females and juveniles on land becomes equal. An extended movement of these frogs is observed in summer evenings especially after rain. During breeding phase, abdomen of females are seen distended and due to enlarged ovaries and occasionally guts are completely empty. Spawns and

larval stages can be observed in nature from April to October at Gauhati and for some what shorter period from May to September at Shillong. They are abundantly available during peak rainy months. During breeding phase, in July at Gauhati, a marked frog was observed to move as far as 128 m. from its release point and sometimes beyond the study site. The marked released frogs in April were recaptured in December also showing a definite home range.

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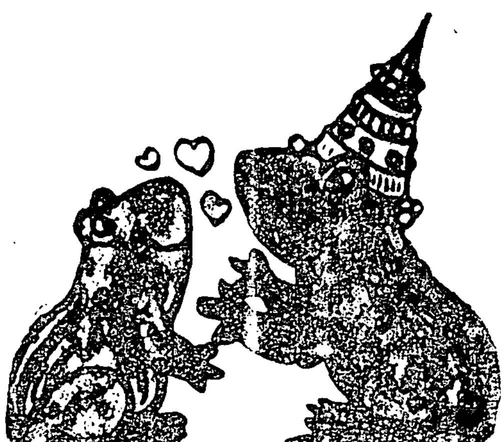
* Original not seen.

CHAPTER – 4

FOOD & FEEDING HABITS



INTRODUCTION

Various workers have investigated the food and feeding habits of anurans from different parts of the world. More important of these, particularly on genus Rana, are Liu and Chen (1932); Smith (1951); Turner (1959); Berry (1965); Itamics and Koskela (1970); Haapanen (1970); Kramck (1972); Khan (1973) Blackith and Speight (1974). Main thrust of these contributions has been to find out the percentage composition of food items taken by different Rana species, seasonal variations and food habits, volume and quality of food intake in different months, prey composition in relation to collection sites, frog food and its abundance in the vicinity and frog's gape in relation to its feeding habit. Many workers have reported frogs as non-selection feeders. Importance of frogs as biological control agents of insect pests of crops is also well known (Liu and Chen, 1932 and Khan, 1973).

In the present chapter food and feeding habits of Rana cyanophlyctis, investigated for its two populations one at Gauhati and another at Shillong have been presented. The investigation deals with percentage composition of the food items present in the gut during breeding season in in detail, a comparison of food volume and percentage composition of food intake during annual breeding cycle and a percentage analysis of food intake during 12 months to find out food performance, and food intake in relation to environmental factors. The information may be useful as prefered items may be cultured on mass scale for maintenance and culture of this species.

REVIEW OF LITERATURE

A survey of literature reveals that following aspects of food and feeding habits of anurans have been investigated by different workers.

Various workers such as Jarnefelt (1915); Smith (1951); Holmes (1959); Goin and Goin (1962); Korschgen and Moyle (1962); Korschgen and Baskett (1963); Janseen and Klimstra (1966); Dickerson (1969) and Hedeen (1970) have described anurans as opportunistic feeders. They noted that the frogs have a tendency to snap and would eat any object that moves and the can swallow. Itamics and Koskela (1970); Blackith and Speight

(1974) explained the presence of large quantity of vegetable materials in the gut content of Rana temporaria. The frogs engulf vegetable material as mistake rather than as routined food. Brugger (1972); Brown (1974), Tucker and Michael (1975) attributed their unsuccessful attempt of swallowing unpalatable venomous toads, to its opportunistic feeding behaviour. While working on Rana tigrina, Khan (1973) noted that it eats inorganic and organic food items and exhibits ravenous and opportunistic feeding habit. Southwood (1966) noted that the relative abundance of organic matter caught by anuran to a large extent dictates the large mobility of ground living organism, the one most active being are most frequently caught. Lescure (1964) observed that Bufo bufo prefer fast prey than slow moving one and preys that scurry rather glides such as slug and earthworm. However, Metter (1964) noticed the variation of diets in the frog collected from different localities. The variation, however, has been the reflection of the predominance of the food observed in such localities. Similarly, analysing the gut contents of the anurans collected from different localities in America Klimstra and Myers (1965) observed that food contents in frogs and toads gut reflected the abundance of taxon of the localities. Berry (1965) analysed the gut contents of Bufo asper, and compared the contents with the availability of taxon in the surrounding, she noted varying percentage of food types in the gut and in the surrounding and concluded that this could be evident of selection of prey, it could have also been due to the difference between the sizes of the toads involved or due to difference between habitat. In 1970 she observed that Bufo asper collected from different localities showed different food items in their gut content analysis, indicating unselective feeding behaviour. Kramck (1972) derived a relationship between different size group and food preference for fast and slow moving preys. The contingency test of association for different size groups showed no significant difference ($P > 0.01$) whereas, the numbers of fast and slow moving prey taken by the frog showed a significant difference ($P < 0.02$). In Rana temporaria Blackith and Speight noted that the limit of prey consumption has been fixed by its own relative immobility and the size of the month gape and the food supply depends on the mobility of the invertebrate species. Theyhave also recorded noxious emperor moth, fox moth, caterpillers the food of the bull frog reflecting its non selective feeding habit. Clarke (1974) made an exhaustive study on the food and feeding type of genus Bufo and recorded that the large size toads sit and wait to consume the passing prey while the small ones and juveniles search for their prey he also mentioned that the ability of toads to eat noxious and armored insect which are abundant has been partly because of their immunity from other predators, ensuring an adequate food supply to the toads for which there is little competition. This ability to utilize the uniquitous taxas alongwith other available arthopods and unpalatability of toad by most of their potential predators has been accountable for the world wide succers of the toad. Labanick (1976) compared the food consumed by the cricket frog to the availability of invertebrates at ground and above ground level and recorded a significant positive correlations which indicated that the prey selection has not been as important as prey availability. He, further noticed that food in the cricket frog (Acris creptians) contained 33% items belonging from above ground, 45% from ground and 2.3% from aquatic level.

Eckert (1934) in toads and Sweetman (1944) in hylids showed preference for arthopod food, whereas Smith and Bragg (1949) noticed that frogs do not have any food preference and the food content reflects the abundance of the taxas of the locality. Bragg (1957) felt that the nature of movement of prey has been an important factor for its capture and believed that it was by this mean that adult toads discriminate between toadlets and vertebrate of same size group to restrict cannibalism. Kaesss and Kaess (1960) noted that apparent motion of the prey has been an essential stimulus to elicit the feeding strike in Bufo terrestris. Commenting on the food preference and competition for it, Inger and Marx (1961) recorded that the size of prey later has little relation to the size of predator, and significant correlations coefficient have been estimated on interspecific comparison of predator and prey size. They concluded that each species has an inherent size range to which it responds, over a wide range of body sizes and further believed that this would reinforce niche separation, since smaller individuals would compete with the adult of their own species rather than those of the different species with a smaller adult size. Berry and Bullock (1962) recorded predominance of the arthropods in the food items of the toad Bufo melanostictus collected from different localities. They noticed that the toad has preference of ground dwelling forms of size ranging from 5.0 mm 20.0 mm and observed that the diet varied in toads collected from different localities. Further, they recorded Bufo melanostictus as an unselective ravenous eater, found eating unpaletable animals with protective and offensive mechanism like scorpion, centripedes and millipedes. Cole (1962) in Bufo alvaris and Browarand Brower (1962) in common European frog, recorded wide variety of food and noticed the abundance of the insect.

As far as anuran food is concerned, workers have reported following food types consumed by them:

Arthropods: The gut content analysis of frogs and toads of different localities invariably showed the predominance of Arthropods specially the class Insecta. Brugger (1972) observed 35 families of Insect from the April, May collection: 28 families from July and August collection and 27 families from September and October collection of frogs. Blackith and Speight (1974) observed eleven orders of Insecta and Clerke (1974) found twelve orders of Insecta from 108 gut content analysis of toads. Khan (1973) analysing the gut content of Rana tigrina observed the predominance of Arthropods and noted it to be 60% of total volume measured. The major food item noted during post and pre-breeding periods were coleoptera (Scarabaidae - 14% Tenabroiondae - 12%) orthoptera (Acridae - 24% Grylhdae - 10%). The insect orders commonly recorded in food were coleoptera (adult and larvae); lepidoptera (larvae); Trichoptera (adult and larvae); Placoptera; Hymnoptera; Ordonata; Orthoptera; Hemiptera; Diptera; Neuroptera (larvae) Mecoptera (larvae); Dermoptera and Trichoptera. The other Arthropods often seen are Isopoda; Decapoda; Chilopoda; Diplopoda; and Archnidae. Metter (1964) observed Gastropoda, Spider; Pseudoscropion, ticks and mites, Chilopoda; Diplopoda; Placopteran (adult and nymph); Colembella; Diptera (adult and larvae); coleoptera (adult and larvae); Tricoptera (adult and larvae); Lepidoptera (adult and larvae); Homoptera; Hemiptera; Epheuroptera; Orthoptera; Neuroptem and Inorganic miscellaneous items in food of <u>Ascaphus truci</u>.

<u>Fishes</u>: Frog using fish or its developmental stage as food is not a frequent phenomena, Bruggers (1972); Korschgen and Baskett (1963) have reported small quantity of fish food in stream dwelling frogs, Schonberger (1945) and Aliva and Fyre (1977) in few aquatic anurans. Harold and Bacon (1966) observed that fish were found eating tadpoles of frogs more frequently than tadpoles of toads, may be because of smell or taste. Grubb (1972) observed fish <u>Gamusia affins</u> eating eggs and tadpoles of Bufonoidae, Ranidae and hylidae. Similarly Kurse and Francis (1977) observed that the fish like large mouthbass (<u>Micropterus salmoides</u>): green sunfish (<u>Lapomis cyanellus</u>) and the black bull head (<u>Letalurus meles</u>) ate larvae of the striped chorus frog (<u>Pseudacris triscriata</u>); plain leopard frog (<u>Rana blairi</u>), northern leopard frog (<u>Rana pipiens</u>) and spodifoot toad (<u>Scaphiopus bombifrous</u>). They demonstrated that brass prefers <u>Rana blairi</u> larvae more than <u>Rana catesbeiana</u> larvae. They also noticed that if fishes were provided by sufficient numbers of <u>Rana blairi</u> larvae, they totaly refuse bull frog's

tadpole, and recorded an inverse relationship between larval frog habitat and larval frog palatability. Werschkul and Christensen (1977) observed that the fish blue gill sunfish at significantly more tadpoles than eggs of _Rana sphenocephala_ and _Rana arcolata_ hand have no discrimination feeding pattern for the two anurans tadpoles or their developing embryonic stages. The large amounts of eggs as fish food confirms that the blue gill sunfish do not have preference for mobile or immobile foods.

Cannibalism: Cannibalism are often reported in anurans. McCann (1933) observed _Rana cyanophlyctis_ consuming large quantity of tadpoles. He attributed this to pressing circumstances due to shortage of food. Khan (1973) observed _Rana tigrina_ eating larvae, juveniles and adults _Bufo stomaticus_, small _Rana tigrina_ and _Rana cyanophlyctis_. He further observed that although _Rana cyanophlyctis_ populations were largest, _Rana tigrina_ could rarely caught them due to their agility. Bruggers (1972) observed toads and froglets in the diet of bull frog Berry (1970), Berry and Bullock (1962), Inger and Marx (1961), observed froglets and toads in diet of American frog. Munz (1920) and McCoy (1969) observed _Bufo_ in the gut content of _Rana catesbeiana_, indicating *Bufo*, as the frogs food. However, Don Fulk and Whitaker (1968) and Lewis (1962) observed no *Bufo* in stomachs content of *Rana catesbeiana*, although *Bufo woodhousei* were very common in the area from where *Rana catesbeiana* had been collected for the gut content analysis.

Reptiles: Reptiles as a food of frogs has been rarely recorded. McCann (1933) observed a large size *Rana tigrina* eating Bull stiped keel back snake (*Trophodonatus stocapus*). Korschgen and Moyle (1955) recorded coral snakes, alligator and salmander in the diet of American bull frogs. Similarly Bruggers (1972) observed representative from the family Ambystomidoe as food of a Ohio bull frog.

Birds: McCann (1933) observed a small water bird in the food of Indian bull frog. Grey (1954) noticed a *Rana tigrina* attacking and overpowering Kinger fish. Dharmraju (1960) found a large bull frog attacking Poultry. Dhashpande (1965) noticed a small sparrow in the food of *Rana tigrina*. Khan (1973) from Pakistan reported chick, and nestling sparrow in the food of Indian bull frog. Howard (1950) in American bull frog Rana catesbeiana observed brown towhee *Pipilo fuscus*, and *Geothlypis*.

Mammals: Thus Grey (1954) recorded small mice in *Rana tigrina*.

Banerji (1954) observed Rana tigrina attacking squirell. Cohen and Howard (1958) observed Rabbit pellets on the gut content analysis of American bull frog. Creel (1963) noted Bats in the gut content analysis of Rana pipiens. Bruggers (1972) recorded few rodentia (common rat) in the food of American bull frog. Khan (1973) observed Crocidura pergrisea (common mole), young rat and Rattus novregicus in the food of Rana tigrina from Pakistan.

Plant materials: Apart from animal food numerous vegetative materials have been observed in the gut content analysis of anurans. Witson (1955) observed Polygonum flowers and leaves of Marinda lucida in the food of Bufo regularis. Tyler (1959) observed Polygonum flowers and glass blades in the food of Rana esculanta. Berry and Bullock (1962) observed buds, seeds, leaves and twig, apart from large quantity of conifer needles in the gut of Bufo melanostictus. He believed that the large quantity could not have been due to misidentification but because of its feeding habit. Berry (1965) in Surgapon toads, Turner (1959) in Rana pipien pretiosa, Korschgen and Baskett (1963) in stream dwelling bull frog, Hedeen (1970) in mink frog and Stewart and Sandison (1972) in mink frogs, green frog and bull frog recorded large quantity of plant materials. Stanley (1972) noticed that 90.5% frogs of the total frog examined for gut content in Rana septentrionalis showed the presence of vegetative materials. Out of total food intake Itamics and Koskela (1970) reported 15% of total food as plant material in Rana temporaria and Khan (1973) noticed 13% in Rana tigrina. The vegetative materials constituted mainly Ellodea leaves, dry and green glass blades; filaments of Spirogira and few unidentified leaves and buds. Itamics and Koskela (1970) and Blackith and Speight (1974) explained that the presence of the vegetative material is due to the opportunistic feeding habit and injesting falling leaves in mistake of food. Korschgen and Baskett (1963) and Hedeen (1970) felt that wind caused floating of plant materials which must have triggered the feeding response in frogs.

Inorganic and Miscellaneous: Miscellaneous substance like Pebbles (Ezekel 1931); Feathers stones, and hairs (Cohen and Howard (1955) Debris, stone and mud (Berry and Bullock (1962) and small pieces of rope, small pieces of brick, rolls of human hair, rolls of cattle dung are recorded in gut content analysis of various anurans. Khan (1973) measured it to be 9% of total food of Rana tigrina.

Diet in relations to Size Variation and Seasonal Changes: Berry and Bullock, (1962) in Malayan toads; Metter, (1964) in tailed frogs; Linzey, (1967) in Rana pipiens; Hedeen (1970) in Rana septentrionalis and Blackith and Speight

(1974) in Rana temporaria observed different diets in anuran populations collected from different localities, and noted it in accordance to the availability of taxa at different sites. Smith, (1947); Savage, (1961); Itamics and Koskela (1970) and Blackith and Speight, (1974) in Rana temporaria observed little if at all, feeding during their breeding months and active feeding during the pre and post spawning periods. Khan (1973) divided annual breeding periods of Rana tigrina in four type (1) Pre breeding period ranging from late February to June, and noticed food to be predominated by coleopteran and orthopterar (2) Breeding period, ranging from July to mid September which is marked by the rise in atmospheric temperature, humidity and rainfall and subsequently increase in various insect fauna, however, shows low feeding intensity (3) Post Breeding period ranging from late September to late November where the atmospheric conditions are same with little fluctuations, showed ravenous feeding behaviour. At this stage the surrounding contained varieties of Insect fauna. (4) Hibernation period ranging from late November to early February marked by low insect fauna and low feeding. At this stage the site and food mainly show coleoptran and orthoptern. He further observed that rise in temperature increased the feeding activity of the frog and maximum feeding was recorded on clear moon nights. During reproductive phase the male stops feeding first and croaks followed by female fasting for reproduction. Brooks (1964) felt that the difference in diet of Rana catesbeiana is due to different size, sex and habitat of the frogs. Berry (1965) mentioned that feeding in anurans has been related to the ecological conditions. Khan (1973) felt Physiological factors to be more responsible than ecological one. In Rana temporaria, Blackith and Speight (1974) observed maximum food intake during June and recorded 127 items/animal. He further observed that anuran feeding is correlated with seasonal fluctuation and the availability of insects. 60% of the Rana temporaria collected during winter months had empty stomach. Commenting on the nutritive value of food, Berry and Bullock (1962) observed that the food value depends on the percentage which had been digested and utilized by the toads. A rough indication of the useful food can be obtained by knowing the amount of prey recovered from the rectum. He further, mentioned that the food parts usually not utilized are head, Elytra and other such exoskeleton. In Diptera, spiracle and caphalo-pheryngeal skeletons had been two major indigestive parts recovered from rectum. Itamics and Koskela (1970) mentioned that molluscan can be caught more easily or preferred more than beetles because of its slow motion and more food value. Blackith and Speight (1974) noted that the most abundant prey were coleopterans however interms of wet body mass moluscans are the most important.

Specialized feeding behaviour has been reported in various anuran. McCann (1933) noted that Rana cyanophlyctis secure prey from both above and water surfaces. The food mainly consist of Insect, little frogs and large amount of tadpoles, and are reported as important animal for the destruction of tadpoles. Commenting on the fooding habit of metamorphosing froglets, he mentioned that young frogs soon after meta mophoss leave the water and set under rock and stone for 3 - 4 days without feeding after which they eat ravenously. He also recorded in adult frogs the use of fore-legs to hasten large size meals. Schuierer (1961) and Livezey (1961) observed that the toad Bufo boreas of the deep spring valley in California are diurnally active and showed preference for the aquatic food. Aliva and Fyre (1977) have also noted that the aquatic feeding behaviour in anurans are hypothesized to be highly specialized mechanism.

Holmes (1959) mentioned that the frogs and toads have the power of ejecting out indigestable bodies from their stomachs and small grass piece or moss accidentally swallowed with the food were vomitted out. Nigam (1977) observed the vomitting capabilities in Rana tigrina and Rana cyanophlyctis. Blackith and Speight (1974) observed that Rana temporaria adopts an exceedingly sedentary habit while feeding i.e. why predominance of ground living organism were noticed in its food. Moreover, the frog does not predate on aquatic organism (except at times when encounter out of water) as it is incapable of capturing prey in water. He also observed that it helps in determining the relative abundance of different organism in the frog gut and it acts independently of the ecological factors, indicating the relative abundance of these organism in the ecosystem. He noted that *Rana temporaria* consume a disproportionately high numbers of invertebrates predators and thereby exerts major influence on energy flow in the ecosystem. Ashby (1969) remarked Poikilothermy is also advantageous in anurans, because the frog metabatic activity becomes lowest at a time of year when prey is both less active and less common. Cochran (1968) noticed that the American toad in captivity ate 9,936 insects in 3 months; a marine toad 53 mosquitoes in 1 min; *Rana* catesbriana, an enormous amount of insects in few hours. He further recorded this being why large import of anurans specially *Bufo marinus* is made to various North American agricultural countries and recorded that the value of single one to North American farmer, in controlling the inroads of harmful insects has been variously estimated and found varying from 20 to 50 dollars a year. He also remarked that its economical value would be much greater in insect ridden tropical countries.

MATERIAL AND METHODS

The gut contents of Rana cyanophlyctis were analysed every month for one year. Altogether 87 males, 55 females and 92 juveniles of this species from Shillong site, and 78 males, 45 females and 126 juveniles from Gauhati site were then sacrificed for gut content analysis. They were preserved in 10% formalin solution. The body wall of the animals was slit to allow quick penetration of the formalin in the internal organs. For examination of food items, the whole gut of the animal was cut open and divided into 3 parts, stomach, intestine and rectum and contents of each part of the alimentary canal were placed in a petridish. The various food items were sorted out with or without the aid of a binocular microscope.

Before analysing the gut contents, the S.V. length, maximum width, the animal weight, total weight of alimentary canal, the weight of empty alimentary canal (the difference of two weights give the weight of gut content). Its head length, total hind limb and rectum length were recorded. The volume of the food content in the various parts of the alimentary canal were poured in 5 ml of 50% ethenol in graduated cylinder. The amount of liquid displacement was noted as volume of the food content.

Gastro-somatic index

The gut of each frog including the food content was weighed and then cut open longitudinally all along its length. Its contents were removed and then the empty guts were also weighed. From the difference between the weight of total gut and empty gut, weight of food content was found out. The gastro-somatic index for each frog was calculated by taking the ratio of the weight of its food bulk and body weight. The formula used was

$$\text{Gastro-somatic index} = \frac{\text{Weight of food content}}{\text{Weight of animal}} \times 100$$

The coefficient correlation between gastrosomatic index and S.V. length/weight of frog and between gastro-somatic index and weight of food bulk have been calculated.

The bivariate and multivariate correlation coefficient between various types of food consumed by *Rana* *cyanophlyctis* and environmental conditions such as atmospheric temperature, aquatic temperature, humidity rainfall and limnological conditions have also been derived for technique (see Chapter II).

OBSERVATIONS

Every month 19 to 21 frogs were collected at random at Gauhati and Shillong. They were sacrificed and preserved in 10% formalin and their gut contents were analysed. Following aspects were investigated :

1. Nature of food items
2. Percentage composition
3. Annual feeding intensity
   (a) Volume of food intake
   (b) 12 months percentage analysis
4. Food and feeding habits in relation to the environmental conditions.
5. Gastro somatic index.

(1) Nature of Food items

The food items were taken out from the entire gut. Majority of identifiable items were retrived from the stomach. In the intenstine and rectum, the food items were seen distorted and semi-digested. The following items were recorded in the gut contents.

(i) Animals : Insects belonging to order Homoptera, Diptera, Coleoptera, Formisoidea, Hymenoptera, Orthoptera, Odonata, Dictyoptera, Trichoptera, Neuroptera and other items belonging to Arachnida, Oligocheata, Chilopoda, Mollusca, Pisces and Anura. In the Shillong frogs, few additional items, belonging to Plecoptera, Phasmida, Dermaptera, Isoptera, Aphididae and small crabs were also recorded. In one case a small lizard was also found.

(ii) Plants : Algae, twigs, leaves (grass blades) seed and flowers. In Shillong specimens, conifer needles were also recorded.

(iii) Miscellaneous : Mud, stone, coal pieces, cowdung, pebbels, thread feathers and hairs.

There was no difficulty in identifying complete or partially broken items. The difficulty was faced in identifying semidigested items of intestine and rectum. Arthopods, mollosc shell and bony remains of vertebrates were identified with the help of hard parts food. The insects predominated the food items and were identified mainly with the help of head structure, wings, ovipositors and legs or other exoskeletal structures. The beetles were identified with the help of leathery elytra; dipterans by mouth parts and halteres; odonates by wing marking and reticulation; Hymnopterans by first pair of abdomen pubescence and hind tarsi, Homoptera and Heteroptera by mouth parts and wings, orthoptera by hind leg, prothorax and typical wings, Formicoidea by meso and metathorax and thoracic structure Dictyoptera by head structure and wings colour, Trichoptera and Neuroptera by antenna, head shape, body and wing structure; Phasmida with the help of walking legs and body shape, Dermaptera by cerci (Scleroitised in forcep shape) and semi circular hind wing. Spiders were identified by chelicerae, 6 paris of cephalothoracic appendages and pedicels; Molluscs by hard shell; Millipedes and centipedes by head, abdomen and number of walking legs; Oligocheata by metamerically segmented vermiform-body or by chitellum; Crustacea (small crabs) by typical carapace, rostrum and chelate legs. Various vertebrate orders by hard

Fig. 4.8 : Adult female eating adult male. A maximum prey size record in gut content analysis.

Fig. 4.8

exoskeleton and bony structures. The plant materials were characterized in the broad group like, algal parts, twigs, leaves, seed and flowers. The unidentified semidigested inorganic and organic parts and debris were placed under miscellaneous items.

Occasionally in field and in captivity both while swallowing *Rana cyanophlyctis* were observed pushing large size food items with fore limb. In the pond waters of state fish farm Gauhati, during rainy season the tadpoles of *Rana cyanophlyctis* were observed clinging and chewing dead fish frys and spawns.

Among Gauhati frog during January 7 insect types and 11 other types of food items were recorded. During summer months they were 10 and 14 respectively. In December again the number of food types went down to 6 and 12 respectively. (Fig.4·7).

In Shillong frogs during January 5 insect types and 8 other food types were recorded. During summer months they were 10 and 13 respectively. During December again the number went down to 6 and 8 respectively like that of January (Fig. 4.7).

(2) Percentage composition

The average percentage composition of different food items taken by Gauhati frogs was found in following order. Homoptera, 12.47%; Insect larvae and Nymph, 11.66%; Formicoidae, 9.83%; Arachnidae, 8.65%; Coleoptera, 8.42%; Diptera, 6.25%; Mites, 3.76%. The other food items were present in much smaller quantity.

The average annual percentage of food items taken by Shillong frogs was in following order: Insect larvae, 28.42%; Diptera, 16.32%; Homoptera, 11.25%; Coleoptera, 9.12%; Arachnida, 7.00%; Formicoidea, 6.56%; Hymnoptera, 2.39%; Mites, 3.34% and leaves 1.73%. The average annual percentage of other food items was very low.

Comparison between the percentage composition of major groups of food items of the two populations has been illustrated in histograms (Fig. 4.1). The major groups of food items were Arthropods, vegetable matters invertebrates and verebrates. Out of Arthropods, insect formed very high percentage ranging from above 45% of the total food content in January at Shillong to about 70% in May. From June onward their percentage fluctuated between 37% to about 60% in November. In December they formed hardly 25%. In these

Table 4.1

Percentage composition of various food types in Rana cyanophlyctis of Gauhati population

Food Item	Months												Annual food intake(%)
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	
Homoptera	14.89	18.75	13.82	17.02	12.93	9.00	6.69	8.20	7.14	11.93	14.20	18.58	12.47
Hymnoptera	1.06	-	1.59	2.65	0.86	2.00	1.23	2.07	2.55	2.05	2.84	0.64	1.83
Heteroptera	2.65	1.78	2.12	3.31	4.31	2.50	3.08	2.05	3.57	2.05	1.70	1.92	2.54
Diptera	3.19	6.25	8.51	12.76	4.31	4.50	6.79	8.20	7.14	5.76	3.97	2.56	6.25
Coleoptera	5.31	1.78	5.85	7.97	11.20	16.50	12.35	12.30	6.12	14.52	11.36	4.48	8.42
Orthoptera	0.53	5.35	2.12	1.59	2.58	3.00	6.17	2.56	1.02	1.64	-	-	2.07
Odonata	-	-	-	-	-	1.00	0.61	0.51	-	-	-	-	0.18
Formicidae	2.65	11.66	4.78	2.65	13.79	21.50	7.40	14.80	9.69	13.16	8.52	7.05	9.83
Dictyoptera	-	-	-	1.59	-	0.50	-	0.51	-	-	-	-	0.23
Tricoptera & Neuroptera	-	-	-	1.06	0.86	1.00	1.23	1.53	-	-	-	-	0.47
Arachmidae	20.21	13.39	5.85	5.31	3.44	4.00	6.17	6.15	9.69	11.93	5.11	2.17	8.65
Mites	4.25	1.78	2.65	5.31	7.75	-	2.46	1.53	3.06	6.58	1.70	8.97	3.76
Algae	-	-	-	2.12	4.31	1.00	7.40	4.10	7.65	2.88	1.13	-	2.58
Twig	1.06	0.89	4.25	1.06	0.86	1.50	4.32	2.05	3.06	0.82	5.11	4.48	2.44
Leaves	2.65	4.46	5.85	1.59	1.72	3.50	8.02	8.20	10.71	2.88	18.18	5.76	6.21
Seed	-	-	0.53	-	1.13	1.00	0.86	-	1.02	1.02	2.46	-	0.84
Flower	-	-	-	-	-	1.00	2.58	0.61	1.02	-	0.41	-	0.42
Mud	1.59	4.46	3.72	6.38	0.86	1.50	3.70	2.00	4.08	1.64	3.97	5.12	3.20
Stone	1.06	4.46	5.85	5.31	1.72	1.00	1.85	2.05	3.57	0.82	4.54	3.84	2.91
Annelida	0.53	0.89	1.59	0.53	0.76	1.50	2.46	1.02	0.51	2.88	0.56	2.56	1.36
Chilopods	1.59	-	0.53	0.53	1.72	0.50	3.08	1.02	1.53	1.23	1.70	1.28	1.22
Anuran Tadpoles	2.12	8.92	2.12	10.63	3.44	2.00	0.61	1.02	1.02	2.88	-	1.28	2.82
Anuran Subadults	1.06	-	10.10	4.25	-	0.50	0.61	1.53	-	1.23	0.56	3.20	3.29
Insects larvae	13.82	5.35	10.63	1.06	1.72	6.00	3.70	4.10	3.57	5.76	1.13	9.61	5.64
Insects nymph	12.76	6.25	5.85	5.31	10.34	6.50	4.93	7.17	1.53	0.17	1.70	5.12	6.02
Fish	0.53	3.57	1.06	-	-	1.00	1.23	0.51	-	0.41	-	0.64	0.61
Mollusca	-	-	0.53	-	-	0.50	3.08	2.05	7.14	0.41	5.11	0.64	2.61
Miscellaneous	-	-	0.53	0.53	-	0.50	-	2.05	3.57	0.41	-	1.91	0.83

Table 4.2

Percentage composition of various food types in Rana cyanophlyctis of Shillong population

Food Item	Months												Annual food intake(%)
	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	
Homoptera	11.86	9.35	16.14	18.50	10.19	6.39	4.34	6.31	11.26	12.74	14.42	7.65	11.25
Heteroptera	0.84	1.43	3.93	4.84	6.79	1.91	2.53	1.57	1.09	-	2.98	0.51	2.25
Diptera	30.50	24.46	15.74	14.53	7.76	7.00	7.97	10.00	18.46	22.54	25.87	12.75	16.32
Coleoptera	3.38	0.71	10.62	11.01	35.43	5.09	10.14	17.36	2.47	1.96	4.47	1.53	9.12
Formicidae	1.69	4.31	11.41	8.37	2.42	5.09	6.88	4.21	5.21	7.84	9.45	4.59	6.56
Hymnoptera	-	-	4.33	6.16	2.42	0.95	2.53	3.15	1.64	0.98	2.98	0.51	2.39
Dictyoptera	-	-	-	0.44	0.97	-	-	-	0.27	0.49	0.49	-	0.23
Orthoptera	-	0.71	-	0.88	2.91	0.63	0.72	0.52	-	-	-	-	0.55
Odonata	-	-	0.78	0.44	0.97	0.95	1.81	0.52	-	-	0.49	-	0.59
Placoptera	-	-	-	-	-	0.31	0.36	-	-	-	-	-	0.07
Phasmida	-	-	-	0.44	-	-	0.36	1.57	-	-	-	-	0.19
Dermoptera	-	-	-	-	-	-	-	1.05	-	-	-	-	0.03
Isoptera	-	-	1.18	-	0.97	-	-	-	-	-	-	-	0.19
Neuroptera	-	-	-	-	-	-	0.36	-	0.27	-	0.99	-	0.15
Insects larvae	19.49	13.66	9.84	5.28	3.88	53.18	44.92	10.00	35.77	33.33	5.97	48.46	23.21
Insects nymph	1.69	8.63	7.87	5.28	5.33	6.68	4.72	2.67	3.57	1.47	5.97	3.06	5.03
Mites	4.23	4.31	1.96	3.52	0.97	5.09	1.08	1.57	3.57	2.45	7.46	2.04	3.34
Arachmidae	5.08	21.58	6.29	8.81	4.85	1.91	4.34	5.78	10.04	6.86	9.95	5.61	7.00
Leaf	1.69	1.43	0.39	0.88	0.97	-	1.08	12.10	0.54	0.98	1.49	1.02	1.73
Twig	0.84	1.43	-	0.44	1.45	0.32	0.72	11.05	0.27	0.98	0.99	2.04	1.57
Algae	1.69	2.87	3.14	3.96	5.33	-	-	3.68	1.09	2.94	1.99	3.06	2.59
Mudroll	10.16	2.15	-	1.32	-	0.32	-	0.52	0.54	1.96	2.48	4.08	1.77
Stone	0.84	0.72	0.40	2.20	-	0.96	-	1.10	7.55	-	0.51	0.51	0.79
Coal piece	-	-	0.79	-	-	-	-	0.53	0.27	-	-	-	0.16
Seed	-	-	-	-	-	-	-	-	0.27	0.49	-	-	0.15
Miscellaneous (Hair, feather etc.)	-	-	-	-	-	-	-	-	0.27	-	0.99	-	0.11
Flower	-	-	-	-	-	-	-	0.27	-	-	0.49	-	0.07
Annelida	-	-	0.78	0.44	1.94	0.32	-	0.53	-	-	-	-	0.39
Arthropods	-	-	1.18	-	1.45	0.64	-	0.53	-	-	-	-	0.35
Chilopods	-	1.43	1.18	0.44	-	-	-	-	0.27	-	-	-	0.28
Aphids	5.93	-	1.96	1.76	-	-	-	2.11	-	1.96	0.49	2.55	1.19
Anuran tadpoles	-	-	-	-	0.97	-	-	0.53	-	-	-	-	0.16
Fish	-	-	0.72	-	-	1.27	-	-	-	-	-	-	0.19
Mollusca	-	-	-	-	0.49	0.32	0.36	0.27	-	-	-	-	0.11

months when their percentage was very low i.e. 25% in June, 35% in July and 25% in December, the percentage of their larval stages intake was very high, bring about 60%, 50% and 50% respectively during the same months. Though the insect larvae were present through out the year their highest percentages in January, July and December coicides with their high breeding activities of insect in these months. It is well known that June and July are the breeding months of all kinds of insect, the high percentage in December included mostly Chironomus and other aquatic Dipteran larvae.

In Gauhati populations the percentage of insect went up to 60% in June, upto 70% in August about 50% in October, but percentage of insect larvae doe never as high as in Shillong population. Availability of higher percentage of insect during these months coincides with monsoon and post monsoon months, when the insect population in nature was also very high. Low accurrence of insect larvae in the food items in comparison to that of Shillong population may be attributed to periodically cleaning and insecticides spraying of the fishery ponds.

Other items, seems to form very low percentage through out the year both in Gauhati as well as Shillong populations.

Fig. 4.1 : Percentage composition of various food types recorded in the gut content analysis, during 1978 in <u>Rana cyanophlyctis</u> from Gauhati and Shillong populations.

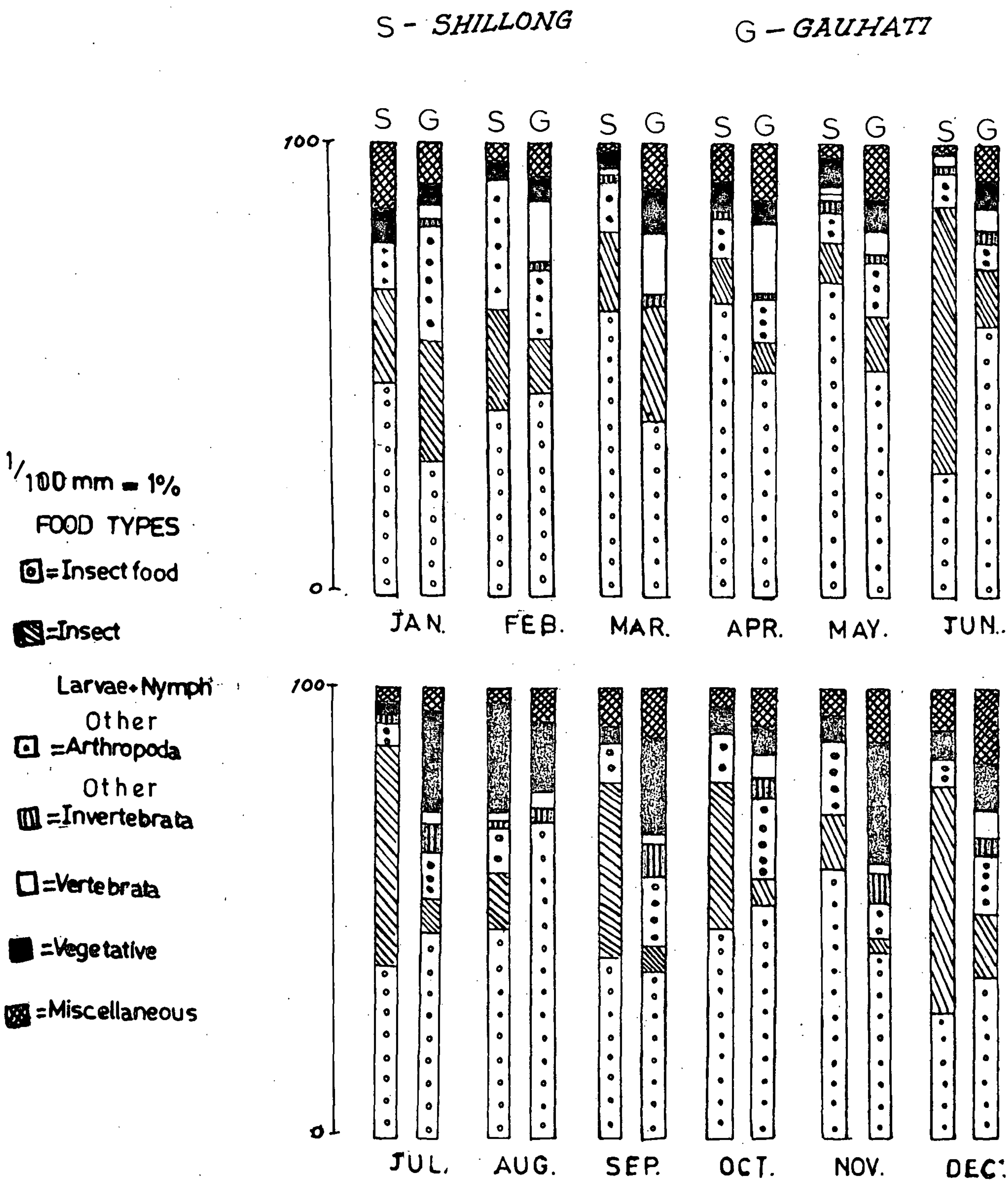

S - SHILLONG
G – GAUHATI
S G
100
0
JAN.
FEB.
MAR.
APR.
MAY.
JUN.
JUL.
AUG.
SEP.
OCT.
NOV.
DEC.
1/100 mm = 1%
FOOD TYPES
=Insect food
=Insect Larvae+Nymph
Other =Arthropoda
Other =Invertebrata
=Vertebrata
=Vegetative
=Miscellaneous

FIG. 4·1

(3) Annual feeding intensity

A. Body length, gut length and food volume

Data on body dimensions, gut length and food volume of *Rana cyanophlyctis* has been presented in Tables 4.3, 4.5 and 4.7 (for Shillong frogs) and 4.4, 4.6, and 4.8 (for Gauhati frogs). Following account deals with a description of these aspects in juveniles, and adult males and females during pre breeding, breeding and post breeding periods.

Shillong population

1) Pre breeding period (Table 4.3)

Juvenile : The S.V.L. of juveniles ranged from 3.4 to 5.1 cm ($\bar{X}$ = 4.0 cm); body weight 4.0 - 16.0 gm ($\bar{X}$ = 8.38 gm); total gut length 10.0 - 13.5 cm ($\bar{X}$ = 10.73 cm); and food volume 0.4 - 3.0 ml ($\bar{X}$ = 0.52 ml).

Males : The S.V.L. varied from 4.0 to 4.5 cm ($\bar{X}$ = 4.25 cm); body weight 7.2 - 11.2 gm ($\bar{X}$ = 9.2 cm); total gut length 11.5 - 12.7 gm ($\bar{X}$ = 12.10 gm); and food volume 0.95 - 1.5 ml ($\bar{X}$ = 1.23 ml).

Table 4.3

Dimension and Volume of Gut Content of Rana cyanophlyctis collected during March from the Shillong site (Sample size - 20).

MEASUREMENTS	MALE		FEMALE		JUVENILES		TOTAL	
	Average	Range	Average	Range	Average	Range	Average	Range
SV length (cm)	4.25	(4.0-4.5)	6.15	(15.9-6.4)	4.00	((3.4-5.1)	4.80	(3.4-64)
Body weight (cm)	9.20	(7.2-11.2)	30.15	(28.5-31.8)	8.375	(4.8-16.0)	15.90	(4.8-31.8)
Body breadth (cm)	1.60	(1.4-1.8)	2.75	(2.4-3.1)	1.62	(1.3-2.4)	1.99	(1.3-3.1)
Head length (cm)	1.45	(1.4-1.5)	2.45	(2.4-2.5)	1.33	(1.2-1.8)	1.74	(1.2-2.5)
T. Hind limb length(cm)	6.45	(6.2-6.7)	10.75	(10.3-11.2)	6.63	(5.4-8.2)	7.94	(5.4-11.2)
Total gut length (cm)	12.10	(11.5-12.7)	19.75	(18.1-21.4)	10.73	(10.0-13.5)	14.19	(10.0-21.4)
Stomach length (cm)	1.40	(1.3-1.5)	2.30	(2.2-2.4)	1.45	(1.2-2.3)	1.71	(1.2-2.4)
Intestine length (cm)	10.20	(9.7-10.5)	16.70	(15.1-18.3)	8.77	(6.3-10.6)	11.89	(6.3-18.3)
Rectum length (cm)	0.60	(0.5-0.7)	0.75	(0.7-0.8)	0.42	(0.3-0.5)	0.59	(0.3-0.8)
Total Food Vol.(ml)	1.23	(0.95-1.5)	2.15	(2.0-2.3)	0.834	(0.4-3.0)	1.40	(0.4-3.0)
Stomach Food Vol.(ml)	0.55	(0.50-0.60)	0.75	(0.7-0.8)	0.52	(0.2-2.4)	0.61	(0.2-2.4)
Intesting Food Vol.(ml)	0.375	(0.35-0.4)	1.275	(1.15-1.4)	0.26	(0.2-0.5)	0.66	(0.2-1.4)
Rectum Food Vol.(ml)	0.06	(0.06)	0.125	(0.1-0.15)	0.142	(0.1-0.2)	0.109	(0.06-0.2)

Females : The SVL of the females varied from 5.9 to 6.4 cm ($\bar{X}$ = 6.15 cm), body weight, 28.5 - 31.8 gm ($\bar{X}$ = 30.15 gm), total gut length 18.1 - 21.4 cm ($\bar{X}$ = 19.75 cm), food volume 2.0 - 2.3 ml ($\bar{X}$ = 2.25 ml).

2) Breeding period (Table 4.5)

Juvenile : The SVL of juvenile varied from 2.4 to 5.1 cm ($\bar{X}$ = 3.89 cm), body weight 5.7 - 20.7 gm ($\bar{X}$ = 10.71 gm), total gut length 11.6 - 17.8 cm ($\bar{X}$ = 14.94 cm), and food volume 0.35 - 0.95 ml ($\bar{X}$ = 0.68 ml).

Male : The SVL of males varied from 3.7 to 4.0 cm ($\bar{X}$ = 3.86 cm), body weight 9.0 - 10.0 gm ($\bar{X}$ = 9.5 gm), total gut length 10.9 - 14.8 cm ($\bar{X}$ = 12.95 cm), and food volume 0.5 - 0.95 ml ($\bar{X}$ = 0.75 ml).

Female : The SVL of female varied from 5.6 to 6.0 cm ($\bar{X}$ = 5.8 cm), body weight 24.8 - 36.0 gm ($\bar{X}$ = 29.20 gm), total gut length 19.0 - 20.9 cm ($\bar{X}$ = 19.83 cm), and food volume 1.15 - 3.0 ml ($\bar{X}$ = 2.10 ml).

3) Post breeding period (Table 4.7)

Juvenile : The SVL of the juveniles varied from 3.2 to 5.35 cm

Table 4.4

Dimension and Volume of Gut Content of the *Rana cyanophlyctis* collected during March from the Gauhati site (Sam size - 21).

MEASUREMENTS	MALE		FEMALE		JUVENILES		TOTAL	
	Average	Range	Average	Range	Average	Range	Average	Range
SV Length (cm)	4.3	(4.0-4.6)	5.78	(5.65-5.9)	5.03	(4.6-5.4)	5.04	(4.0-5.9)
Body weight (cm)	9.84	(8.2-13.4)	23.90	(22.1-27.0)	17.42	(12.3-22.7)	16.99	(8.2-27.0)
Body breadth (cm)	1.87	(1.8-2.0)	2.83	(2.6-3.2)	2.15	(1.8-2.7)	2.18	(1.8-3.2)
Head length (cm)	1.60	(1.45-1.8)	2.13	(2.0-2.2)	1.85	(1.7-2.1)	1.86	(1.45-2.2)
T. hind limb length(cm)	6.72	(6.1-7.9)	9.70	(8.4-10.7)	8.53	(7.4-9.1)	8.31	(6.1-10.7)
Total gut length (cm)	9.07	(7.3-12.0)	14.3	(11.0-16.0)	13.98-	(13.3-15.6)	12.45	(7.3-16.0)
Stomach length (cm)	1.47	(1.4-1.6)	1.96	(1.6-2.2)	2.00	(1.4-2.5)	1.81	(1.4-2.5)
Intestine length (cm)	7.125	(5.5-10.2)	12.23	(8.9-14.4)	11.55	(10.7-12.6)	10.30	(55.5-14.4)
Rectum length (cm)	0.35	(0.1-0.4)	0.46	(0.4-0.5)	0.43	(0.4-0.5)	0.41	(0.1-0.5)
Total Food Vol.(ml)	0.86	(0.45-1.1)	0.93	(0.6-1.45)	1.36	(0.9-2.0)	1.05	(0.45-2.0)
Stomach Vol.(ml)	0.55	(0.2-0.7)	0.46	(0.25-0.85)	0.72	(0.4-1.2)	0.58	(0.2-0.85)
Intestine Vol.(ml)	0.34	(0.2-0.6)	0.36	(0.25-0.45)	0.48	(0.4-0.7)	0.39	(0.2-0.7)
Rectume Vol.(ml)	0.06	(0.05-0.1)	0.10	(0.05-0.15)	0.13	(0.1-0.2)	0.09	(0.05-0.2)

($\bar{X}$ = 4.15 cm), body weight 4.7 - 18.76 gm ($\bar{X}$ = 8.64 gm), total gut length 10.2 - 14.3 cm ($\bar{X}$ = 11.47 cm) and food volume 0.9 - 1.7 ml ($\bar{X}$ = 1.52 ml).

Male : The SVL of male varied from 3.9 to 4.7 cm ($\bar{X}$ = 4.35 cm), body weight 7.6 - 11.9 gm ($\bar{X}$ = 10.10 gm), total gut length 11.6 - 12.9 cm ($\bar{X}$ = 12.80 cm) and food volume 0.9 - 1.6 ml ($\bar{X}$ = 1.20 ml).

Female : The SVL of female varied from 6.0 to 6.4 cm ($\bar{X}$ = 6.2 cm), body weight 28.60 - 32.80 gm ($\bar{X}$ = 30.70 gm), total gut length 19.50 - 20.3 cm ($\bar{X}$ = 19.90 cm) and food volume 2.14 - 2.38 ml ($\bar{X}$ = 2.26 ml).

Gauhati population

1) Pre breeding period (Table 4.4)

Juveniles : The SVL of the juveniles varied from 4.6 - 5.4 cm ($\bar{X}$ = 5.03 cm), body weight 12.3 - 22.7 gm ($\bar{X}$ = 17.42 gm) and total gut length 13.3 - 15.6 cm ($\bar{X}$ = 13.98 cm), and food volume 0.9 - 2.0 ml ($\bar{X}$ = 1.36 ml).

Males : The SVL of the males gut content varied from 4.0 to 4.6 cm ($\bar{X}$ = 4.3 cm), body weight 8.2 - 13.4 gm ($\bar{X}$ = 9.84 gm), total gut length 7.3 - 12.0 cm ($\bar{X}$ = 9.07 cm) and food content from 0.45 - 1.1 ml ($\bar{X}$ = p.86 ml).

Table 4.5

Dimension and Volume of Gut Content of the *Rana cyanophlyctis* collected during June from the Shillong site (Sample size - 20)

MEASUREMENTS	MALE		FEMALE		JUVENILES		TOTAL	
	Average	Range	Average	Range	Average	Range	Average	Range
S.V. length (cm)	3.86	(3.7-4.0)	5.77	(5.6-6.0)	3.89	(2.4-5.1)	4.50	(3.7-6.0)
Body weight (cm)	9.50	(9.0-10.0)	29.20	(24.8-36.0)	10.71	(5.7-20.7)	16.40	(9.0-36.0)
Body breadth (cm)	1.85	(7.7-2.0)	2.93	(2.7-3.2)	1.82	(1.4-2.5)	2.2	(1.4-3.2)
Head length (cm)	1.40	(1.3-1.5)	2.0	(1.8-2.3)	1.45	(1.2-2.0)	1.6	(1.3-2.3)
T. Hind limb length (cm)	5.825	(5.4-6.3)	9.13	(8.9-9.3)	6.21	(5.0-8.9)	6.9	(5.0-9.3)
Total gut length (cm)	12.95	(10.9-14.8)	19.83	(19.0-20.9)	14.94	(11.6-17.8)	15.9-	(10.9-20.9)
Stomach length (cm)	1.425	(1.2-1.6)	2.73	(2.1-3.1)	1.62	(1.2-2.3)	1.52	(1.2-3.1)
Intestine length (cm)	10.675	(9.2-12.5)	16.30	(16.0-16.8)	13.73	(9.5-16.9)	13.2	(9.2-16.9)
Rectum length (cm)	0.85	(0.5-1.2)	0.80	(0.6-1.0)	0.85	(0.5-1.1)	0.83	(0.5-1.2)
Total food Vol. (ml)	0.725	(0.5-0.95)	2.10	(1.15-3.0)	0.68	(0.35-0.95)	1.33	(0.35-3.0)
Stomach Food Vol.(ml)	0.30	(0.25-0.4)	1.27	(0.65-1.6)	0.29	(0.15-0.35)	0.82	(0.15-1.6)
Intestine Food Vol.(ml)	0.225	(0.05-0.3)	0.65	(0.4-1.1)	0.27	(0.15-0.4)	0.38	(0.05-1.1)
Rectum Food Vol.(ml)	0.10	(0.05-0.1)	0.183	(0.1-0.3)	0.12	(0.05-0.2)	0.13	(0.05-0.3)

Females : The SVL of the female varied from 5.65 to 5.9 cm ($\bar{X}$ = 5.78 cm), body weight 22.1 - 27.0 gm ($\bar{X}$ = 23.90 gm), total gut length 11.0 - 16.0 cm ($\bar{X}$ = 14.3 cm) and food volume from 0.6 - 1.45 ml ($\bar{X}$ = 0.93 ml).

2) Breeding period (Table 4.6)

Juveniles : The SVL of juvenile varied from 4.8 to 5.4 cm ($\bar{X}$ = 5.18 cm) body weight 16.4 - 18.8 gm ($\bar{X}$ = 18.02 gm), total gut length 12.2 - 16.3 cm ($\bar{X}$ = 13.90 cm) and food volume 0.5 - 0.7 ml ($\bar{X}$ = 0.61 ml).

Male : The SVL of male varied from 4.0 to 4.3 cm ($\bar{X}$ = 4.13 cm), body weight 8.95 - 10.1 gm ($\bar{X}$ = 9.66 gm), total gut length 10.1 - 11.2 cm ($\bar{X}$ = 10.88 cm), and food volume 0.4 - 0.55 ml ($\bar{X}$ = 0.49 ml).

Female : The SVL of female varied from 5.6 to 6.45 cm ($\bar{X}$ = 5.89 cm), body weight 22.0 - 33.8 gm ($\bar{X}$ = 27.23 gm), total gut length 15.1 - 22.0 cm ($\bar{X}$ = 17.17 cm), and food volume 0.4 - 2.45 ml ($\bar{X}$ = 0.86 ml).

3) Post breeding period

Juveniles : The SVL varied from 4.3 to 5.2 cm ($\bar{X}$ = 4.6 cm),

Table 4.6

Dimension and Volume of Gut Content of the *Rana cyanophlyctis* collected during June from the Gauhati site (Sample size - 21)

	MALE		FEMALE		JUVENILES		TOTAL	
	Average	Range	Average	Range	Average	Range	Average	Range
S.V. length (cm)	4.125	(4.0-4.3)	5.89	(5.6-6.45)	5.18	(4.8-5.4)	5.06	(4.8-6.45)
Body weight (cm)	9.66	(8.95-10.1)	27.23	(22.0-33.8)	18.02	(16.4-18.8)	18.30	(8.95-33.8)
Body breadth (cm)	1.74	(1.65-1.8)	2.37	(2.15-2.6)	1.67	(1.35-2.2)	1.96	(1.35-2.6)
Head length (cm)	1.69	(1.65-1.7)	2.15	(1.8-2.15)	1.90	(1.8-2.0)	1.91	(1.65-2.15)
T. Hind limb length (cm)	6.95	(6.8-7.1)	9.41	(8.9-10.05)	8.62	(7.9-9.3)	8.36	(6.8-10.05)
Total gut length (cm)	10.88	(10.1-11.2)	17.17	(15.1-22.0)	13.90	(12.2-16.3)	10.65	(10.1-22.0)
Stomach length (cm)	1.65	(1.4-1.9)	2.37	(2.0-2.7)	2.08	(1.8-2.2)	2.00	(1.4-2.7)
Intestine length (cm)	8.65	(8.4-9.1)	14.27	(12.4-15.2)	11.30	(9.5-13.7)	11.40	(8.4-15.2)
Rectum length (cm)	0.375	(0.3-0.4)	0.64	(0.5-1.0)	0.55	(0.4-0.7)	0.55	(0.3-1.0)
Total food Vol.(ml)	0.49	(0.4-0.55)	0.86	(0.4-2.45)	0.61	(0.5-0.7)	0.65	(0.4-2.45)
Stomach food Vol.(ml)	0.24	(0.2-0.3)	0.41	(0.2-1.20)	0.34	(0.25-0.55)	0.33	(0.2-1.20)
Intestine food Vol.(ml)	0.175	(0.15-0.3)	0.32	(0.15-0.85)	0.24	(0.2-0.25)	0.265	(0.15-0.85)
Rectum food Vol.(ml)	0.625	(0.05-0.1)	0.13	(0.5-0.4)	0.07	(0.5-0.1)	0.007	(0.5-0.4)

body weight 9.85 - 25.8 gm ($\bar{X}$ = 20.4 gm), total gut length 8.95 - 14.45 cm ($\bar{X}$ = 13.98 cm), and food volume 0.95 - 2.05 ml ($\bar{X}$ = 1.45 ml).

<u>Male</u> : The SVL of male varied from 4.2 to 4.9 cm ($\bar{X}$ = 4.6 cm), body weight 9.1 - 11.35 gm ($\bar{X}$ = 10.68 gm), total gut length 8.45 - 10.75 cm ($\bar{X}$ = 9.7 cm), and food volume 0.9 - 1.05 ml ($\bar{X}$ = 0.95 ml).

<u>Female</u> : The SVL of female varied from 5.6 to 6.05 cm ($\bar{X}$ = 5.95 cm), body weight 24.6 - 33.05 gm ($\bar{X}$ = 30.05 gm), total gut length 13.8 - 17.15 cm ($\bar{X}$ = 15.25 cm), and food volume 0.9 - 1.35 ml ($\bar{X}$ = 1.20 ml).

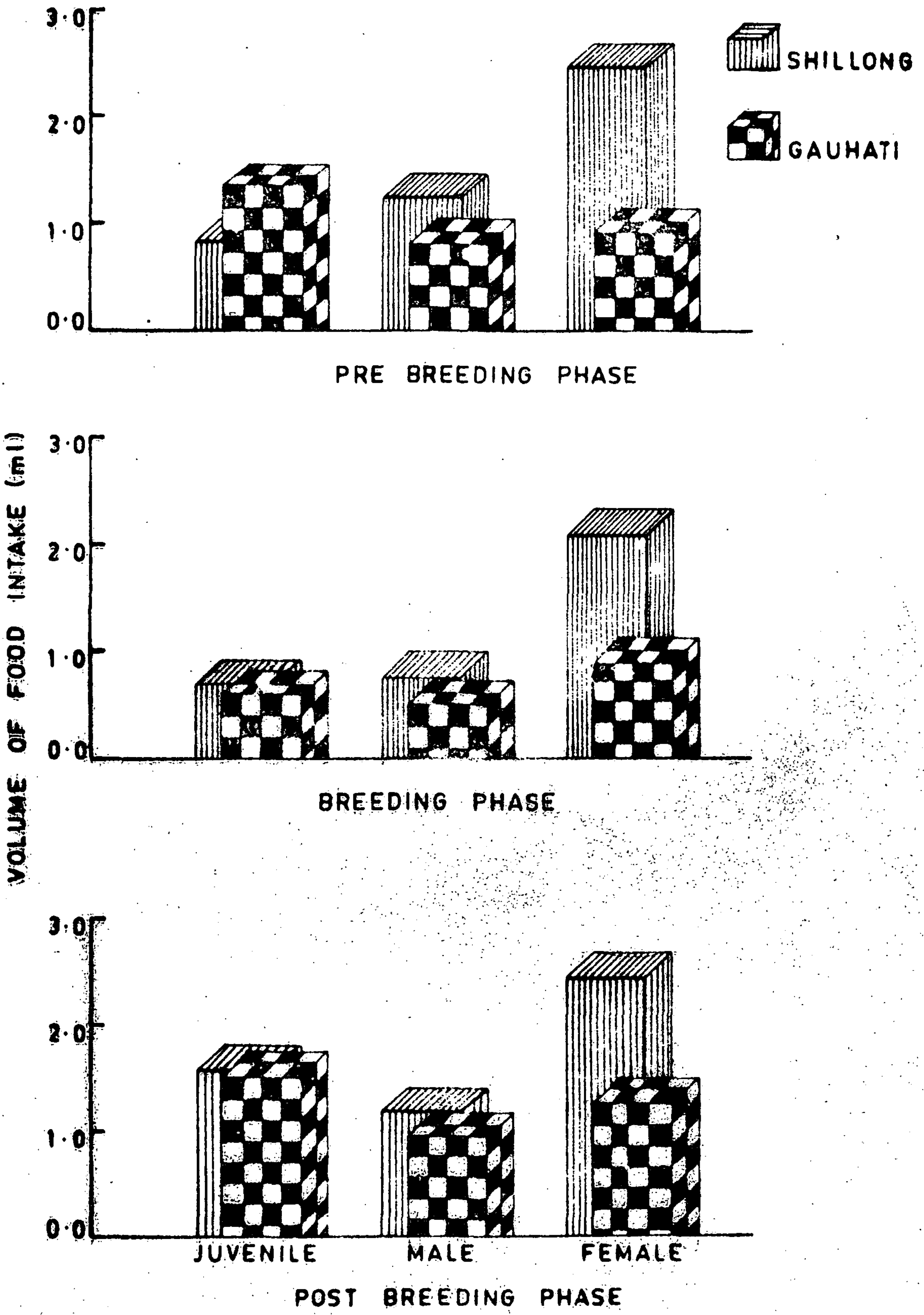
A comparison of the food volume of juveniles, males and females has been illustrated in Fig. 4.2. In an average the food volume of Gauhati frogs was larger than in Shillong frogs. It is somewhat low in both populations in the breeding period in comparison to other periods. It may be due to somewhat larger size of the ovary. Few females were observed with empty stomachs. This phase of empty may be very short duration. As soon as spawning occur the size of ovary is reduced and frogs again start eating voraceously.

Table 4.7

Dimension and Volume of Gut Content of *Rana cyanophlyctis* collected during October from the Shillong site (Sample size - 20).

MEASUREMENTS	MALE		FEMALE		JUVENILES		TOTAL	
	Average	Range	Average	Range	Average	Range	Average	Range
S.V. length (cm)	4.35	(3.9-4.7)	6.2	(6.0-6.4)	4.15	(3.2-5.35)	4.38	(3.52-6.4)
Body weight (cm)	10.10	(7.6-11.9)	30.70	(28.60-32.80)	8.64	(4.7-18.76)	16.70	(4.7-32.80)
Body width (cm)	1.70	(1.45-1.9)	2.90	(2.5-3.2)	1.80	(1.50-2.0)	2.08	(1.45-3.2)
Head length (cm)	1.50	(1.4-1.6)	2.50	(2.35-2.6)	1.40	(1.30-1.50)	1.76	(1.30-2.6)
T. Hind limb length(cm)	6.35	(6.1-7.0)	10.90	(10.4-12.1)	6.90	(5.9-8.7)	7.89	(5.9-12.1)
Total gut length (cm)	12.80	(11.6-12.9)	19.90	(19.50-20.30)	11.47	(8.2-14.3)	12.52	(10.20-20.30)
Stomach length (cm)	1.45	(1.3-1.6)	2.30	(2.2-2.4)	11.50	(0.5-0.40)	1.10	(0.5-2.4)
Intestine length (cm)	10.40	(9.2-10.9)	17.10	(16.1-19.0)	0.9	(7.0-10.2)	9.21	(7.0-19.1)
Rectum length (cm)	0.65	(0.55-0.8)	0.8	(0.7-0.9)	0.45	(0.3-0.55)	0.65	(0.3-0.9)
Total food Vol.(ml)	1.20	(0.9-1.6)	2.26	(2.14-2.38)	1.52	(0.9-1.7)	0.88	(0.9-2.38)
Stomach food Vol.(ml)	0.65	(0.6-0.75)	0.8	(0.7-0.9)	0.9	(0.4-1.1)	0.54	(0.4-1.1)
Intestine food Vol.(ml)	0.45	(0.4-0.6)	1.32	(0.92-1.7)	0.4	(0.35-0.55)	0.33	(0.35-1.7)
Rectal food Vol.(ml)	0.1	(0.88-0.14)	0.14	(0.13-0.15)	0.16	(0.1-0.25)	0.07	(0.8-0.25)

Fig. 4.12 : Volume of food intake during annual breeding cycle of <u>Rana</u> <u>cyanophlyctis</u> of Gauhati and Shillong populations



SHILLONG
GAUHATI
3·0
2·0
1·0
0·0
PRE BREEDING PHASE
VOLUME OF FOOD INTAKE (ml)
BREEDING PHASE
JUVENILE
MALE
FEMALE
POST BREEDING PHASE

FIG. 4·2

Table 4.8

Dimension and Volume of Gut Content of *Rana cyanophlyctis* collected during October from the Gauhati site (Sample size - 20)

MEASUREMENTS	MALE		FEMALE		JUVENILES		TOTAL	
	Average	Range	Average	Range	Average	Range	Average	Range
S.V. length (cm)	4.6	(4.2-6.9)	5.95	(5.6-6.05)	4.6	(4.3-5.2)	4.96	(4.2-6.05)
Body weight (cm)	10.68	(9.1-11.35)	30.05	(24.6-33.1)	20.4	(9.85-25.8)	19.98	(9.1-33.15)
Body width (cm)	1.8	(1.7-2.0)	2.95	(2.5-3.3)	2.0	(1.7-2.45)	2.16	(1.7-3.3)
Head length (cm)	1.5	(1.3-1.7)	2.3	(2.0-2.4)	1.7	(1.5-2.1)	1.50	(1.3-2.4)
T. Hind limb length(cm)	6.3	(5.9-7.7)	9.9	(8.7-10.9)	8.8	(7.6-9.4)	8.20	(5.9-10.9)
Total gut length (cm)	9.7	(8.45-10.75)	15.25	(13.8-17.15)	13.98	(8.95-14.45)	13.59	(8.45-17.15)
Stomach length (cm)	1.55	(1.5-1.6)	2.4	(1.7-2.6)	2.1	(1.40-2.3)	1.87	(1.4-2.6)
Intestine length (cm)	7.95	(6.7-10.5)	12.65	(8.7-15.1)	10.05	(8.9-11.4)	11.00	(6.7-15.1)
Rectum length (cm)	0.3	(0.15-0.5)	0.51	(0.4-10.7)	0.3	(0.1-0.6)	0.51	(0.1-0.7)
Total food Vol.(ml)	0.45	(0.9-1.05)	1.20	(0.9-1.35)	1.45	(0.95-2.05)	1.10	(0.9-2.05)
Stomach food Vol.(ml)	0.6	(0.4-0.8)	0.52	(0.4-0.65)	0.75	(0.5-1.35)	0.58	(0.4-1.35)
Intestine food Vol.(ml)	0.45	(0.4-0.5)	0.44	(0.4-0.5)	0.52	(0.4-0.75)	0.44	(0.4-0.75)
Rectal food Vol.(ml)	0.1	(0.05-0.15)	0.15	(0.1-0.2)	0.14	(0.1-0.25)	0.66	(0.5-0.25)

B. 12 months analysis

The pattern of annual fluctuation of different types of food items is following.

Gauhati population (Fig. 4.4, 4.3)

Gauhati frog population showed a higher percentage of insects in their food items throughout the year. The percentage of major food items was recorded as follows.

Homoptera: It constituted highest percentage of food items throughout the year, varying from 14.89% in January, 6.79% in July to 18.58% in December with an average annual intake of 12.47%.

Formicidae: The percentage composition of the formicidae varied from 2.65% in January, 21.50% in July to 7.05% in December with an average annual intake of 9.83%.

Arachmida: The percentage composition of Arachnida varied from 20.21% in January, 4.00% in June to 2.17% in December with an average annual intake of 8.65%.

Coleoptera: The percentage composition of Coleoptera varied from 1.78% in February, 16.50% in June to 4.48% in December with an average annual intake of 8.42%.

Fig. 4.3: Percentage variation of Insect food types recorded in the gut content analysis of *Rana cyanophlyctis* from Gauhati population during 1978.

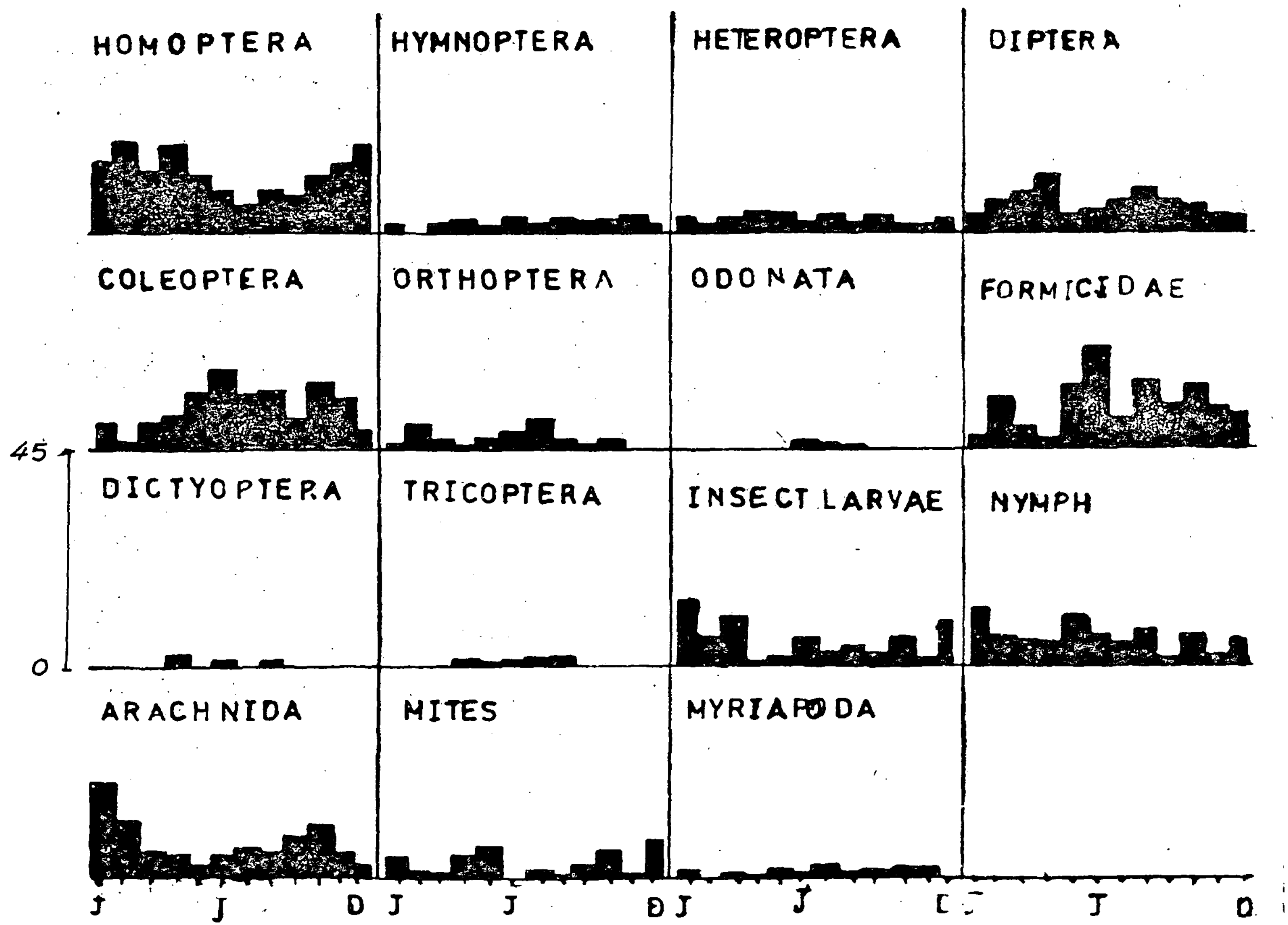

HOMOPTERA
HYMNOPTERA
HETEROPTERA
DIPTERA
COLEOPTERA
ORTHOPTERA
ODONATA
FORMICIDAE
45
DICTYOPTERA
TRICOPTERA
INSECT LARVAE
NYMPH
0
ARACHNIDA
MITES
MYRIAPODA
J J D J J D J J D J J D

FIG. 4·3

Fig. 4.4 : Percentage variations in other food types (except insects) recorded during gut content analysis of <u>Rana cyanophlyctis</u> from Gauhati populations during 1978.

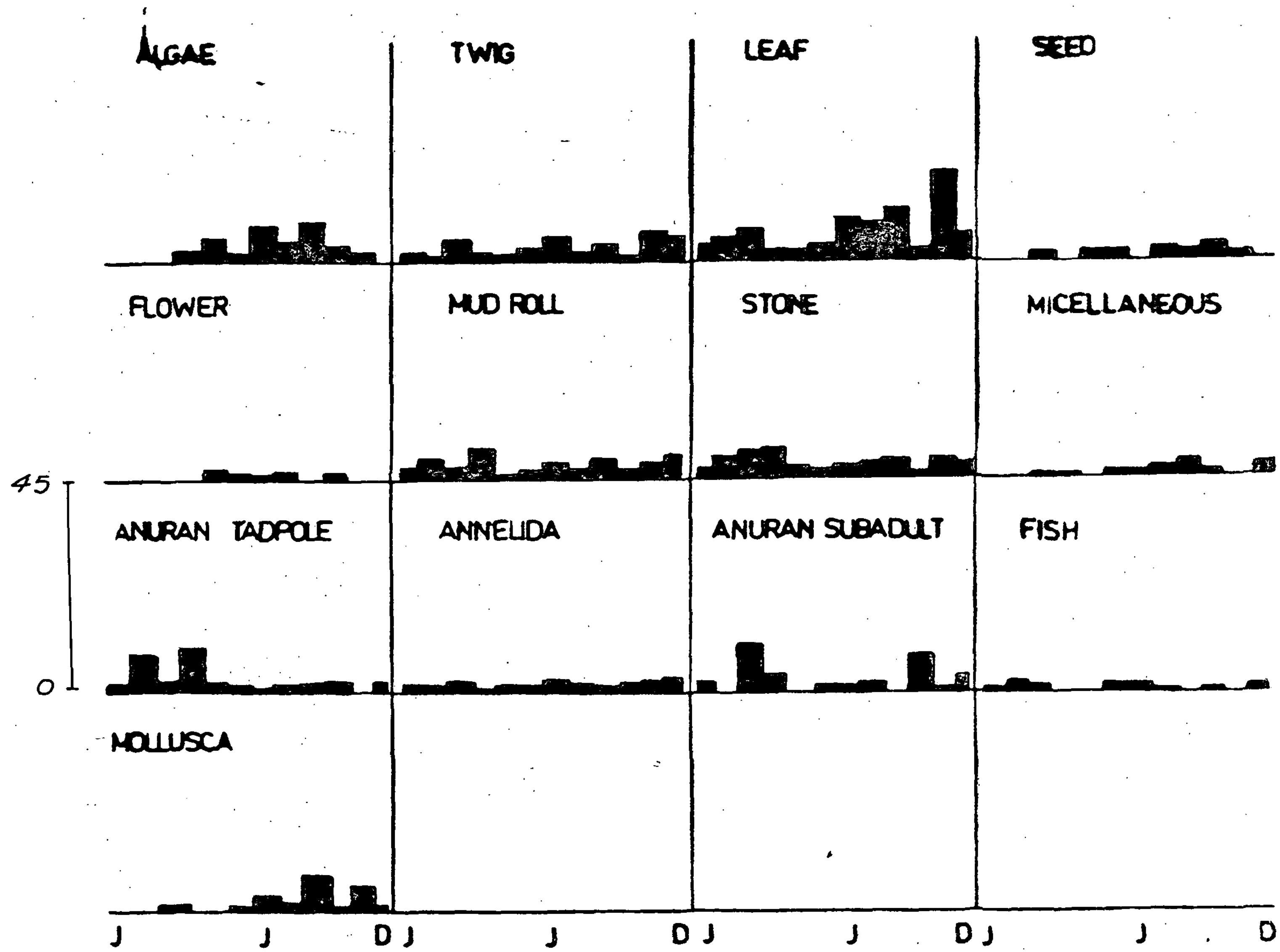

ALGAE
TWIG
LEAF
SEED
FLOWER
MUD ROLL
STONE
MICELLANEOUS
45
0
ANURAN TADPOLE
ANNELIDA
ANURAN SUBADULT
FISH
MOLLUSCA
J
J
D
J
J
D
J
J
D
J
J
D

FIG. 4·4

Diptera: The percentage composition of Diptera varied from 3.19% in January, 12.76% in April to 2.56% in December with an average annual intake of 6.25%.

Leaves: The percentage composition of leaves varied from 2.65% in January, 1.72% in May to 18.18% in November with an average annual intake of 6.21%.

Insect nymph: The percentage composition of insect nymph varied from 12.76% in January, 6.5% in June to 1.70% in November with an average annual intake of 6.02%.

Insect larvae: The percentage composition of insect larvae varied from 13.82% in January, 1.06% in April to 9.61% in December with an annual average of 5.64%.

The percentage composition of Hymnoptera, Heteroptera, Myriapoda, Annehida, Anura, Twigs and other miscellaneous items was very low (0.53% to 4.3%) and showed little fluctuation in 12 month analysis.

Shillong population (Fig. 4.4 & 4.5)

Shillong population also showed a higher percentage of insect in their food items throughout the year. The percentage of major food items were recorded as follows.

Fig. 4.5 : Percentage variations in insect food only recorded during gut content analysis of *Rana cyanophlyctis* from Shillong population during 1978.

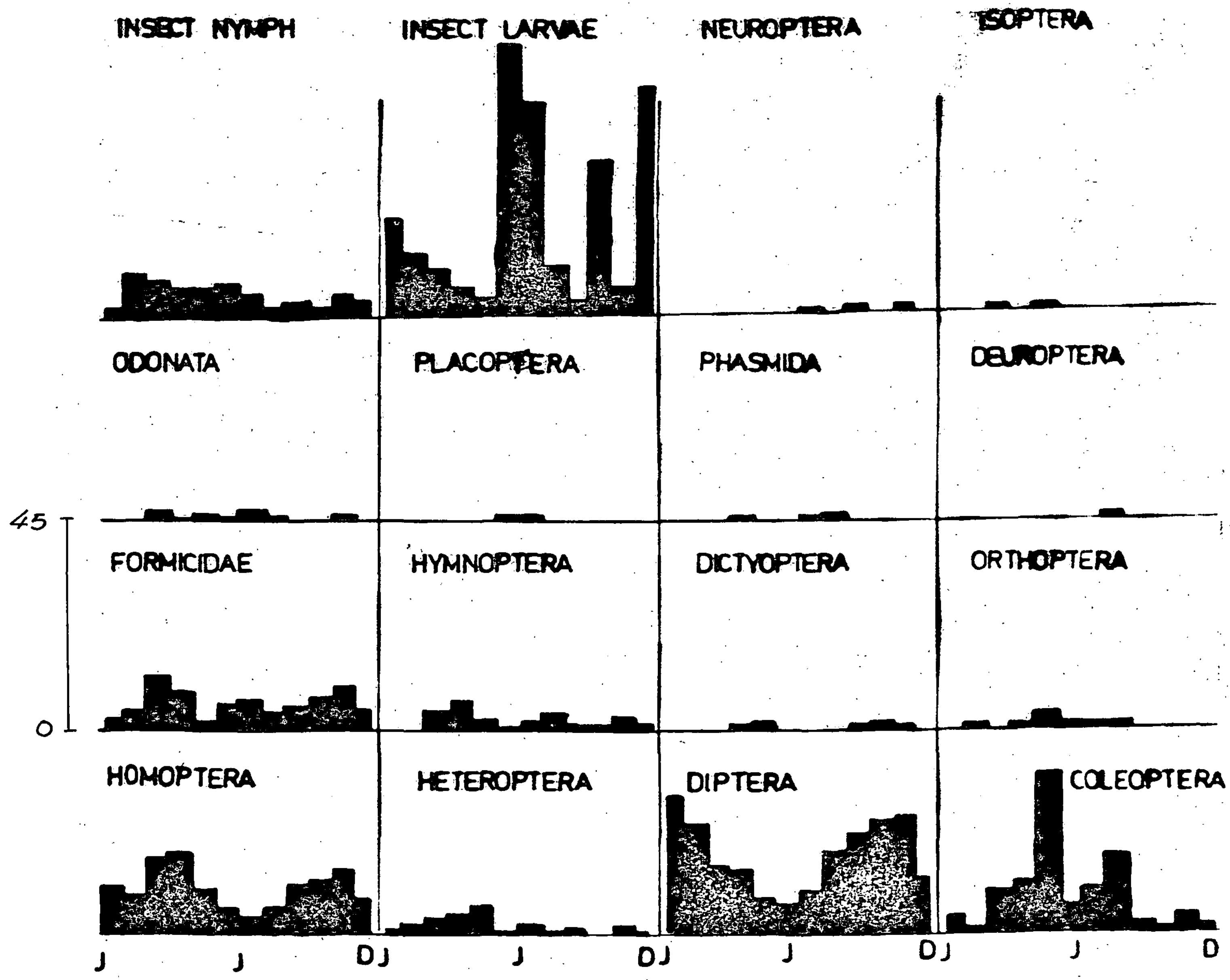

INSECT NYMPH
INSECT LARVAE
NEUROPTERA
ISOPTERA
ODONATA
PLACOPTERA
PHASMIDA
DEUROPTERA
45
0
FORMICIDAE
HYMNOPTERA
DICTYOPTERA
ORTHOPTERA
HOMOPTERA
HETEROPTERA
DIPTERA
COLEOPTERA
J
J
DJ
J
DJ
J
DJ
J
D

FIG. 4·5

Insect larvae: It constituted the highest percentage and was recorded as major food item throughout the year. The percentage varying from 19.49% in January, 53.18% in June and 48.46% in December with an average annual intake of 23.21%.

Diptera: The percentage composition of Diptera in food varied from 30.50% in January, 7.0% in June to 12.75% in December with an average annual intake of 16.32%.

Homoptera: The percentage composition of Homoptera in food varied from 11.86% in January, 4.34% in July to 14.42% in November with an average annual intake of 11.25%.

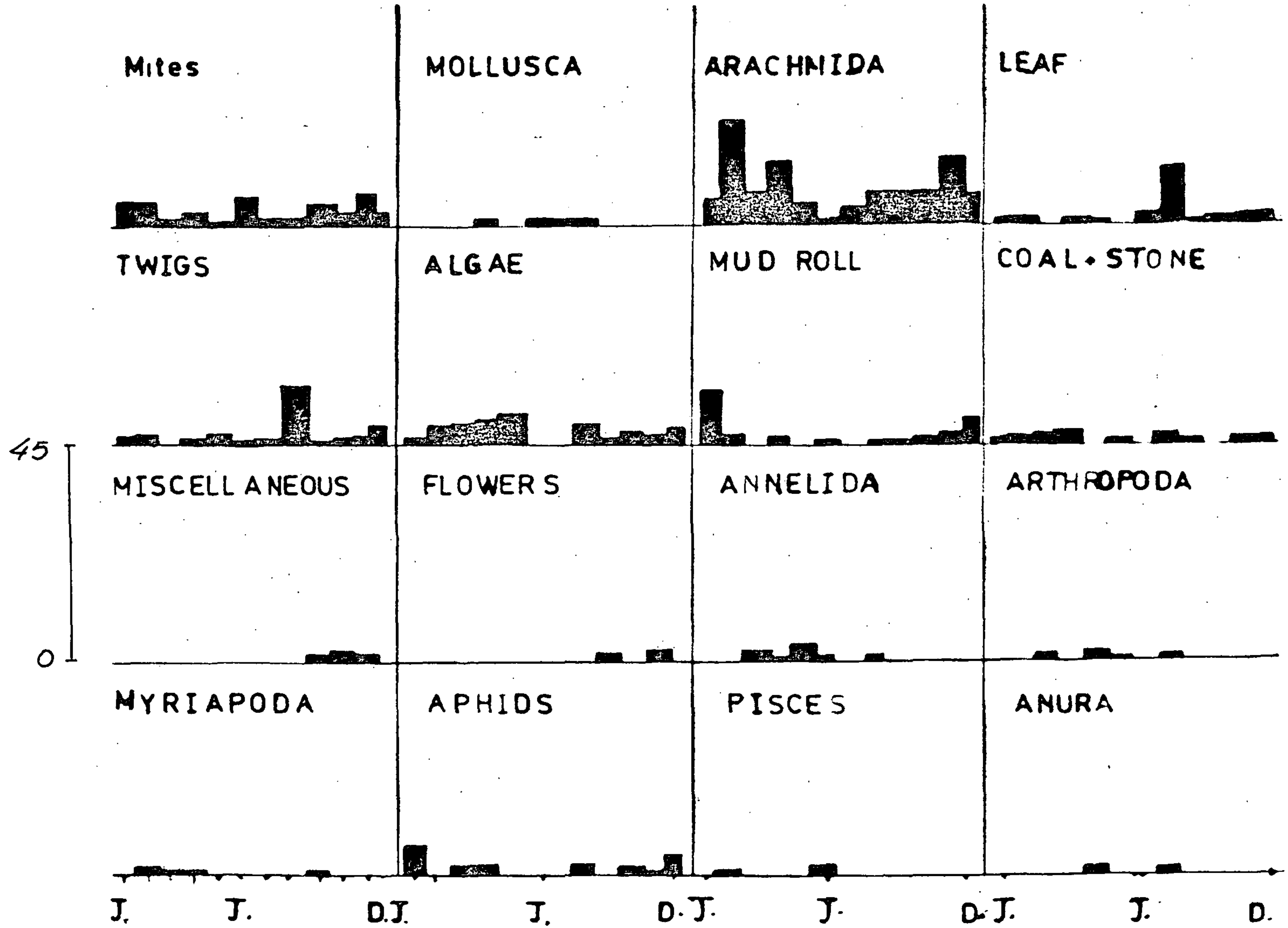
Coleoptera: The percentage composition of Coleoptera in food varied from 0.71% in February, 35.43% in May to 1.53% in December with an average annual intake of 9.12%.

Arachmida: The percentage composition of Arachmida in food varied from 21.58% in February, 1.91% in June to 5.61% in December with an average annual intake of 7.00%.

Formicidae: The percentage composition of Formicidae in food varied from 1.68% in January, 11.41% in March to 4.59% in December with an average annual intake of 6.56%.

Insect nymph: The percentage composition of Insect nymphs in

Fig. 4.6 : Percentage variation in other food types (except adult insects) recorded during gut content analysis of <u>Rana cyanophlyctis</u> from Shillong population during 1978.



Mites
MOLLUSCA
ARACHNIDA
LEAF
TWIGS
ALGAE
MUD ROLL
COAL + STONE
MISCELLANEOUS
FLOWERS
ANNELIDA
ARTHROPODA
MYRIAPODA
APHIDS
PISCES
ANURA
45
0
J.
J.
D.J.
J.
D.J.
J.
D.J.
J.
D.

FIG. 4·6

food varied from 1.69% in January, 6.68% in June to 3.06% in December with an average annual intake of 5.03%.

Mites: The percentage composition of mites in food varied from 4.23% in January, 0.97% in May to 7.46% in November with an average annual intake of 3.34%.

The percentage composition of other food items were recorded low and the average annual intake varied from 0.11% to 2.59.

On comparison it would be seen that consumption of insect larvae is very high throughout the year in Shillong frogs than in Gauhati frogs. Vegetative materials were in very high percentage in Gauhati frogs. Cannibalism and fish as food was prominently recorded in Gauhati frogs only. In both populations Dipteran, Homopterans, Coleoptera formed the large bulk. From the group Insecta Homoptera was highest in Gauhati frogs though the percentage Dipterans was highest in Shillong frogs.

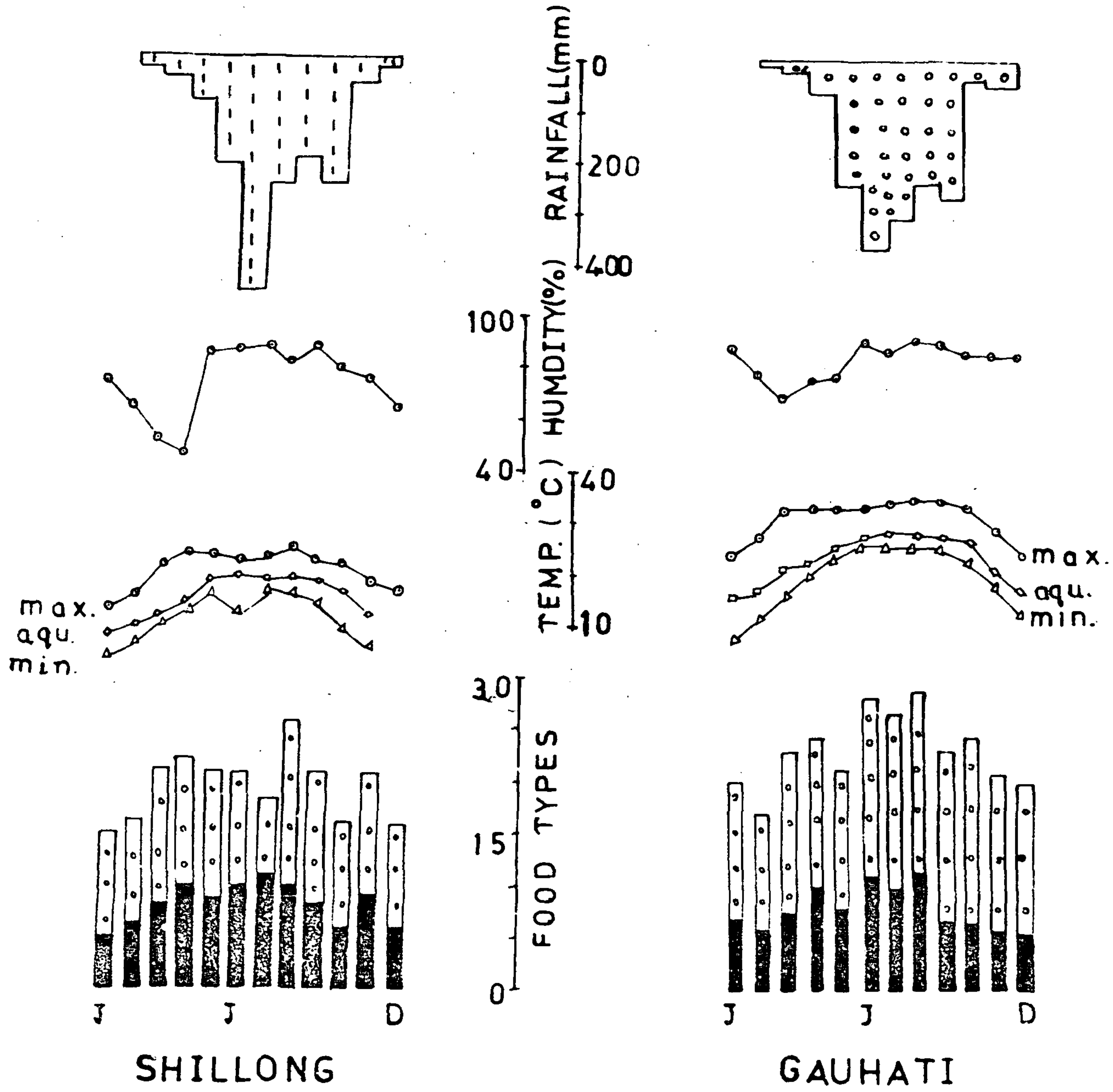
4) Food and feeding in correlation to environmental conditions

The abundance of *Rana cyanophlyctis* on land, at Gauhati and Shillong study sites and subseqently its movement and activities were found to be governed by various factors (see Chapter 3). In the following description, correlation between environmental conditions and food and feeding habit of the frog at the two sites, Shillong and Gauhati have been derived.

Climatic conditions : The correlation and correlation coefficient of aquatic temperature, atmospheric temperature, relative humidity and total rainfall have been worked out separately for different food items. The multiple correlation have also been derived. The data has been compiled and presented in Table 4.9.

Significant correlation coefficient was derived for molluscan food items with aquatic temperature ($r = 0.63$, $P < 0.05$), with atmospheric temperature ($r = 0.71$, $P < 0.05$), with humidity ($r = 0.74$, $P < 0.05$), with rainfall ($r = 0.87$, $P < 0.01$). Although Mollusca constitute only 2.6% of total food intake the analysis suggests that it is also a preferred food item at optimum environmental conditions. The other significant correlation coefficient have been found for fish with rainfall ($r = 0.66$, $P < 0.05$). The correlation coefficient (r) of total food intake in different months with atmospheric temperature was 0.69 ($P < 0.05$), and with rainfall 0.73, ($P < 0.05$).

Fig. 4.7 : Quantitative records of different types of food in the gut content analysis of *Rana cyanophlyctis* during different months of 1978 from Gauhati and Shillong populations.



RAINFALL(mm)
0
200
400
HUMDITY(%)
100
40
TEMP. (°C)
40
10
max.
aqu.
min.
FOOD TYPES
30
15
0
J
J
D
SHILLONG
GAUHATI

FIG. 4·7

Among Shillong frog population the significant correlation coefficient found between insect food items and atmospheric temperature ($r = 0.61$, $P < 0.05$), and aquatic temperature ($r = 0.64$, $P < 0.05$); anuran food with rainfall ($r = 0.56$, $P < 0.05$) and total food intake in different month with temperature ($r = 0.52$, $P < 0.05$).

At both the sites, Shillong as well as Gauhati, the temperature has been found to influence the feeding activities of Rana cyanophlyctis as during summer months largest variety of food items were quantitative and qualitative recorded from its gut (see Fig. 4.5). During rainy months number of spawn and fish fry increase tremendously at Gauhati fish dales leading to larger consumption of fish by the frogs, that at any other period. This accounts for significant correlationccoefficient for fish food with rainfall ($r = 0.66$, $P < 0.05$).

Among Gauhati frogs, the significant correlation coefficient in the following bivariates have been observed. pH and insect food ($r = 0.60$, $P < 0.05$). pH and earthropod food ($r = 0.73$, $P < 0.05$), pH and total food consumed ($r = 0.78$, $P < 0.05$). The other correlation coefficient derived were found to be insignificant and showed no relationship with the food types of the ffrog.

Table 4.9

The values of correlation coefficient derived between environmental conditions and food types consumed by *Rana cyanophlyctis* at Gauhati and Shillong sites.

SHILLONG					DEPENDENT VARIABLE	GAUHATI				
MULTIPLE 'R' CORRELATION	RAINFALL 'r'	RELATIVE HUMIDITY 'r'	TEMPERATURE 'r' AQUATIC	TEMPERATURE 'r' ATMOSPHERIC		TEMPERATURE 'r' ATMOSPHERIC	TEMPERATURE 'r' AQUATIC	RELATIVE HUMIDITY 'r'	RAINFALL	MULTIPLE 'R' CORRELATION
0.5545	0.3249	0.4444	0.6417*	0.6085*	INSECT FOOD	0.55016*	0.4629	0.1810	0.1456	0.3569
0.3536	0.4499	0.3236	0.5262	0.3355	VEG.FOOD	0.4298	0.5016	0.1336	0.0360	-
0.5116	0.2174	0.5352	0.4095	0.4500	ARTHOPOD	0.1087	0.0603	0.1527	0.0806	-
0.8145	0.5641*	0.4934	0.1188	0.2305	ANURANS	0.4464	0.5177	0.4121	0.3289	-
0.3328	0.1702	0.0999	0.3039	0.1138	FISH	0.0159	0.0081	0.2312	0.6621*	0.7633
-	0.0374	0.2906	0.3883	0.3451	LARVAE & NYMPH	0.1783	0.0136	0.2665	0.5859	0.7515
-	0.4502	0.3430	0.3936	0.2066	MOLLUSC	0.6357*	0.7139*	0.7498**	0.8736**	0.8877
-	0.1343	0.1255	0.2366	0.1929	MISCELLANEOUS	0.5002	0.4694	0.2052	0.4154	-
-	0.1281	0.0799	0.3319	0.3552	ANNELIDS	0.5357	0.4108	0.0352	0.3866	-
-	0.5374	0.2715	0.3393	0.3321	CHILOPODS	0.1521	0.0696	0.3594	0.0835	-
0.6770	0.3670	0.0216	0.5201*	0.3979	TOTAL FOOD	0.6884*	0.4818	0.1735	0.7296*	0.87661

* - at 5% level
** - at 1% level

Among Shillong frogs the significant correlation coefficient in the following bivariates have been observed, pH and insect food ($r = 0.52$, $P < 0.05$), pH and mollusc food ($r = 0.58$, $P < 0.05$), pH and total food intake ($r = 0.72$, $P < 0.01$). Total hardness and insect food ($r = 0.59$, $P < 0.05$).

<u>Gastrosomatic Index</u> :

Total food intake (Quantitative) in *Rana cyanophlyctis* and its relationship with S.V. length and body weight of the frog has been derived. The data has been compiled and presented in Table 4.10. It would be noted that in both sexes there is no correlation between the gastro somatic index and/or food and S.V. length and/or body weight. The frogs of the same size had different types as well as percentage and showed gastro somatic index. The correlation coefficient derived between S.V. length/weight of the frog and gastro somatic index have been found insignificant confirming no relationship in two varieties and supporting the frog as opportunistic feeder. However, the correlation coefficient derived between gastrosomatic index and total food intake has been found significant at 1% probability level ($P < 0.01$).

Table 4.10

Rana cyanophlyctis : Body length (SVL), weight of frogs and its food and Gastrosomatic Index

MALE				FEMALE			
S.V.L. (cm)	Frog's weight (gm)	Weight of food items (gm)	Gastro somatic index	S.V.L. (cm)	Frog's weight (gm)	Weight of food items (gm)	Gastro somatic index
3.95	4.64	0.1956	4.2235	5.20	20.34	0.0481	0.2364
4.10	7.36	0.1639	2.2255	5.20	17.68	0.6174	3.4960
4.25	8.36	0.3491	4.1768	5.30	18.87	0.0056	0.02968
4.45	12.52	0.2620	2.0927	5.50	18.61	0.0199	0.1069
4.45	9.13	0.1618	1.6322	5.50	18.50	0.4006	2.1646
4.50	12.00	0.5309	4.4242	5.50	19.40	0.2126	1.0959
4.50	11.62	0.0132	0.1136	5.50	17.66	0.3393	1.9211
4.60	10.80	0.2243	2.0768	5.55	17.89	0.5710	3.1905
4.60	12.60	0.2006	1.5920	5.55	18.43	0.6069	3.3071
4.80	14.20	0.6976	4.9127	5.60	22.54	0.0746	0.3310
4.80	13.40	0.2890	2.1567	5.60	18.46	0.4131	2.2379
4.95	16.09	0.0141	0.2851	5.65	21.46	0.4864	2.2660
5.00	12.80	0.0080	0.0648	5.70	23.83	0.1654	0.6928
5.00	16.17	0.0596	0.3685	5.70	19.99	0.4860	2.4315
5.10	15.20	0.2893	1.9033	5.90	24.60	0.5710	2.3218
5.10	17.52	0.2969	1.6940	6.25	27.42	0.8162	2.9761
5.20	18.36	0.2970	1.6177	6.30	26.50	0.7345	2.7715

<u>Comparative analysis at Gauhati and Shillong sites</u>

A comparative analysis of food and feeding of the two populations reveals that :

1. In an annual gut content analysis, the insect belonging to 13 orders were consumed in Shillong frog population and 10 in Gauhati frog population, though in monthly analysis insects belonging to more than 10 orders were never observed at both the places.

2. The percentage of insect food items (adult and developing) was higher in Shillong frog population that at Gauhati frog population. A noticeable high percentage of Dipterans (adult and larvae), predominated by Chironomous larvae, has been observed in Shillong frogs (16.32% and 23.21% respectively) in comparison to that of 6.35% and 5.64% respectively) of Gauhati frogs.

3. The percentage composition of Annelid, Arthropoda, Mollusca, other invertebrata, Vertebrata, vegetation and miscellaneous items was higher in Gauhati frogs than in Shillong frogs.

4. A high consumption of molluscan food and its significant correlation coefficient ($P < 0.05$) with environmental conditions has been observed in Gauhati frogs and not in Shillong frog population (Table 4.9).

5. High cannibalistic incidence was recorded in Gauhati frogs than in Shillong frogs (Table 4.1 & 4.2).

6. The food volume was higher in Shillong frogs than in Gauhati frogs.

DISCUSSION

The predominance of arthropods, particularly insects as food items of anurans has been reported by all workers. The 65% of food items of Rana cyanophlyctis in the present work also was found to comprise insects, belonging to 10 orders in Gauhati population and 13 orders in Shillong. Various other aspects of their food and feeding habits can be discussed as follows :

(1) Opportunistic and unselective feeder:

Frogs have been described as opportunistic feeder i.e. they eat whatever living and moving materials are available and they can catch and swallow (Harnefelt, 1950; Smith, 1951; Holmes, 1950; Goin and Goin, 1962; Koschgen and Moyle, 1962; Koschgen and Baskett, 1963; Janseen and Klimstra, 1966; Dickerson, 1969; Hedeen, 1970; Itamics and Koskela, 1970;

Bruggers, 1972; Brown, 1974; Blackith and Speight, 1974 and Tucker and Michael, 1975). The food analysis of *Rana cyanophlyctis* also indicates that it eats whatever is available in the habitat. Some leaves, mud stones, etc. were also recorded from the gut perhaps because they are swallowed with main food items. Vegetable matter in the diet of anurans has been reported by Witson (1955), Tyler (1958), Berry and Bullock (1962), Berry (1965), Turner (1959), Korschgen and Baskett (1963), Hedeen (1970), Itamics and Koskela (1970), Stewart and Sandison (1972), Stanley (1972), Khan (1973) and Blackith and Speight (1974). Korschgen and Baskett (1963) and Hedeen (1970) felt that the wind caused floating of plant material and thus its movement may trigger a feeding response in the frog. This shows that frogs do not have discriminative power. This may be the reason of the presence of litter pebbles, cattle dung, stone brick, mud, thread, rope pieces, feather and human hair in *Rana cyanophlyctis* as also recorded earlier by Ezakil, 1931; Cohen and Howard, 1958; Berry and Bullock, 1962; and Khan, 1973).

Frogs are also unselective feeders. To mention a few important references, Berry and Bullock (1962) noticed that *Bufo melanostictus* are unselective ravenous eater and devour unpalatable animals such as scorpion, centipedes and millipedes. Clarke (1974) noted that Bufo eats noxious and armoured insects also, which are otherwise believed to be immuned to predators. Similarly Rana temporaria (Blackith and Speight, 1974) and Rana tigrina (Khan, 1973) have been reported to devour unpalatable noxious animals. The abundance of Rana cyanophlyctis throughout Indian Subcontinent may be due to its feeding success on a wide variety of animals similarly as Clarked (1974) has remarked for world wide distribution of toad on same grounds.

(2) Food preference:

Preference of food in relation to size has been observed in various anurans (see Eckert, 1934; Sweetman, 1944; Inger and Marx, 1961; Cole, 1962; Berry, 1965; Greenslade, 1964; Lescure, 1964; Southwood, 1966; Blackith and Speight, 1974). A typical example of anuran food preference has been given by Berry (1970), who found that Bufo asper living in caves fed freely on ants associated with bat guano, but ate few or none of the mites, spiders and Collembola also occurring in the same cave. Further, while studying 5 species of Anura, she found that certain species devoured a much narrower range of food items than others, showing food preference. They may be due to predator habitat and size group. Food habits of skipper frog population at Gauhati and Shillong do show that they are dependent on available insect fauna, prey size and mobility in the vicinity, though prey size has not been thoroughly worked out in the present investigation. Further, a food preference for insect larvae and Dipteran (23.2%) in Shillong frog and Homepteran and Coleopteran in Gauhati frog are recorded. McCann (1933) noted that Rana cyanophlyctis secures prey from both above and below water surface. During the present investigation, slow as well as fast moving food items such as mites, dipteran larvae or flying insects were recorded from its gut. Sagage (1961); Ashby (1969); Blackith and Speight (1974) reported that terrestial frogs comes to land for feeding. Hamilton (1948); Tyler (1958) and Turner (1959) noted that agnatic frogs feed both on land and water preys. The use of fore limb to hasten the large size meal inside the buccal cavity was observed in the present investigation. Earlier McCann (1933) has also reported the use of fore limb in Rana cyanophlyctis, for swallowing large size prey, assisted by muscular contraction that pulls the eye ball down into the roof of the mouth, giving convulsive bink.

(3) Fish as frog food:

Frog eating small fishes and their developing stages is not a frequent phenomena. Schonberger (1945); Korschgen and Baskett (1963) and Bruggers (1972) have recorded small quantity of fish in the food items of frogs dwelling in stream or other water bodies. In the present investigation of fish fry and its developing stages formed 0.19% to 0.61% of food items in Gauhati and Shillong populations of Rana cyanophlyctis. McCann (1933) reported similar behaviour in the skipper frog/tadpoles in Western India.

(4) Cannibalism:

Cannibalic nature of anurans has been reported by McCann (1933); Munz (1920); Inger and Marx (1961); Berry and Bullock (1962); Lewis (1962); McCoy (1969); Don Iulk and Whitaker (1968); Berry (1970); Burger (1972) and Clarke (1974). Cannibalism has been repoorded in the present work also. 6.0% of total annual food intake were pieces of frogs/tadpoles etc whereas monthly analysis showed it to be 10.10% in the food items analysed for March 1978. In Shillong frog population, it was very low, 0.61% of the total food consumed with a maximum of 0.97% during May. Khan (1973) noted cannibalism in Rana tigrina, but discussed that it devours those anuran forms which are less agile and can be caught easily. In the present work high frequency of cannibalism was observed at Gauhati site than at Shillong site presumably because of restricted habitat and availability of its different stages on a large scale.

(5) Seasonal changes and diet:

There are number of reports on diet variations in arurans due to changes of seasons (Smith, 1951; Turner, 1959; Savage, 1961; Berry, 1965; Itamics and Koskela, 1970; Bruggers, 1972; Clarke, 1974; Blackith and Speight, 1974). While working on the annual cycle of Rana tigrina Khan (1973) observed definite variation in feeding types during its 4 annual phases (pre-breeding, breeding, post-breeding and hibernation) and correlated it with the changes in fauna and climatic conditions. He further mentioned that the frogs consume larger amount of food material during pre and post breeding phases than during hibernating and breeding phases. The annual cycle of Rana cyanophlyctis does not show well demarcated phases although on the basis of its breeding pattern its annual breeding cycle is divisible in 3 phases (pre breeding, breeding and post breeding). These frogs do not hibernate and are available in ponds throughout the year. The gut content analysis of male, female and juvenile for all the 12 months in a year showed the presence of various food items indicating active feeding in the population all around the year. Ashby (1969), Savage (1969), Blackith and Speight (1974) have reported variation in feeding intensity of the explosive breeder Rana temporaria during different months. Khan (1973) reported that in Rana tigerina, soon after hibernations and with the rise in atmospheric temperature, it starts feeding actively, and feeding activity is maximum on clear moon nights. However, on the arrival of monsoon the male stops feeding and diverts its concentration and energy on croaking. The female later stops feeding and gets prepared for crouchment. Soon after the sexual activities get over, the two sexes restart eating ravenously till hibernation. Berry (1965) has also recorded reduced feeding in Rana temporaria during breeding phase and explained it as physiological and psycological preparation for its reproductive assignment. Rana cyanophlyctis has a prolonged breeding period (April to October) and breeds with small clutch size (see chapter 6/7). In the present investigation the feeding intensity does not vary much in different annual phases as mentioned above for explosive breeding type Rana tigrina and Rana temporaria. A maximum feeding in Rana cyanophlyctis is observed during summer night soon after rain. The food intake in relation to SV length has been observed to be high in the juveniles of Rana cyanophlyctis than in adult frogs. Clarke (1974) has made similar observation in toads that food consumed by juveniles is more than at adult stages probably because of more energy demands for its growth. The juvenile Rana cyanophlyctis feed ravenously all around the year retreating only for a short duration during extreme cold and hot period.

Berry (1965) recorded that feeding in anurans are related to its ecological conditions. Khan (1943), Blackith and Speight (1974), Koskela and Pasanen (1974) noted that climatic factors like temperature, rainfall and humidity do play an important role in governing the food and feeding of anurans. In the present investigation correlation coefficient in the climatic and environmental condition with various food types and total food intake has been found out (Table 4.9; 4.10). As Rana cyanophlyctis is active all the year round and has prolonged breeding habit, its food and feeding habits are not that much dependent on climatic or environmental conditions. However, with increased rainfall and temperature a significant increase in total food consumption has been observed, and the correlation coefficient derived of food intake with temperature, pH and rainfall ($r = 0.85$; p.85; and 0.63 respectively) were found significant $P < 0.01$. A high Mollusc population at the Gauhati study site has been observed during rainy season. Their presence in food items is statistically significant ($P < 0.05$) with high temperature, rainfall and humidity of rainy season. At both the sites, a neutral pH are recorded during rainy months when the frogs population are most active (see Chapter 3). Significant correlation coefficient ($P < 0.05$) was observed between the total food items, insect as food items and arthropods as food items with the pH of the water bodies at the two sites. Blackith and Speight (1974) recorded that the relative abundance of different organism in the gut of frogs acts independently of the ecological factors, dictating the relative abundance of these organisms in the ecosystem. The food habits of *Rana cyanophlyctis* reflect the presence of those food types in the ecosystem which it eats corroborating the fact that changes in diet are also dependent on the seasonal variation.

Changes in Length and Weight of Gut and Gastro-somatic Index:

Discussing food types and intensity of feeding in various types of toads of United States, Clarke (1974) records "it might be expected that the range of suitable food items would be greater for larger toads and that they would thus consume a greater diversity of items. It might also be expected that females, because of the greater energy demands, would differ from males in the diversity and amount of ingested prey (Schoener, 1969) and the juveniles which are rapidly growing might similarly differ from adult males". In the present investigation a relationship between the weight of different sexes and size groups of frog with corresponding weight of the food consumed has been calculated as gastro somatic index (Table 3). The correlation between the gastro somatic index and weight; and gastro somatic index and SV length both in males and females was found invariably insignificant 'r' = 0.25 at 16 degree of freedom), indicating that there is no correlation between the two. However, correlation coefficient between gastro somatic index and weight of food items was found significant at probability lebel ($P < 0.01$). Further, gastro somatic index varied in the same sex, size and weight groups of the frog have presumably been due to its opportunistic feeding behaviour. Clarke (1974) while working on american toads and Blackith and Speight (1976) on Rana temporaria derived similar conclusion with Mann-Whitney 'U' test and alalysis.

Juszczyk (1950, 1959, 1966); Juszczyk et al. (1966) and Zamachowski (1970) noted marked changes in weight and length of alimentry canal of the explosive breeding types such as Rana esculanta and Rana temporaria during their annual cycles. In the present investigation little variation was noted in the weight and length of alimentry canal of Rana cyanophlyctis during different phases of the annual breeding cycle presumably because it has prolonged breeding habit.

So far as consumption of a disproportionately higher number of invertebrates, predominantly insects is concerned it is likely that through the consumption of these animals the anurans exert further that poikilothermy is advantageous to anuran as the frog metabolism and activity is at lowest at the time of the year when prey is both less active and less common as also investigated in this work.

Comparative analysis at Gauhati and Shillong sites:

Analysing the gut contents of *Bufo asper* from different localities and environmental condition Berry (1965, 1970) recorded remarkable differences in the diet of toads from two population. Similarly Blackith and Speight (1974) have also recorded differences in diet compositions of *Rana temporaria* from different habitats in the west of Ireland. In the present investigation the gut content analysis of the two frog populations shows that the feeding intensity (food intake in ml) and percentage of food items in Gauhati and Shillong frogs has little variations. Blackith and Speight (1974) remarked that the frog will ingest almost all moving object that approaches within its reach and the list of prey identified on the gut content analysis indicates the availability and relative mobility of the prey in the locality.

SUMMARY

This chapter deals with gut content analysis, percentage composition of various food items and amount of food intake throggh annual breeding cycle of *Rana cyanophlyctis* at Gauhati and Shillong sites. Changes in the diet, gastrosomatic index and gut length and weight have been analysed in relation to changes in environmental conditions. The highest percentage of food items in Gauhati frogs was composed of Homoptera 12.47% and Shillong frog, Insect larvae 23.21%. The diet in order of preference consisted of Homoptera, Insect larvae, Formicoidea, Arachmida, Coleoptera and Diptera in Gauhati frog and Insect larvae, Diptera, Homoptera, Coleoptera, Arachmida and Formicoidea in Shillong frog. The overall order of food preference at the two sites did not show much variation. The food volume which indicates the food intake ranged from 0.49 ml to 2.25 ml, during different months.

The food content reflected the food abundance in the ecosystem, clearly in relation to the changing environmental condition during different period of the year. Significant correlation of insect food with temperature and rainfall ($P < 0.05$) was estimated during summer rainy period. The length and weight of alimentary canal did not show much variation and gastro somatic index did not show any significant correlation with SV length or weight of the frog.

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CHAPTER – 5

NORMAL TABLE OF DEVELOPMENT

INTRODUCTION

Normal table of development is a foremost necessity for embryological investigation of any species. Agarwal and Niazi (1977) commented that they facilitate exact determination of specific stage(s) at which any investigation is carried out, without which it is very difficult to properly evaluate and varify the results of different investigations and meaningful conclusions.

Among vertebrates, amphibians have been found to be convenient material for analysis of development. Normal tables are now available for a number of amphibians and many of these have been included in laboratory guides and manuals (e.g. Hamburger, 1947; Rugh, 1962). Certain recent laboratory manuals such as New (1966) and Billett and Wild (1975) include normal table of *Xenopus laevis* of Nieuwkoop and Faber (1967) as it is used as a common laboratory material in many western laboratories. This description has been based on external as well as internal morphological changes.

Among anuran species available in India, developmental tables have been prepared for *Rana tigrina* by Agarwal and Niazi (1977), *Rana limnocharis* by Roy and

Khare (1978), Bufo anderoni by Satyalal and Niazi (1977) and Rhacophorus nigropelmatus by Sahu (1981, unpublished). Though Rana cyanophlyctis has a very wide distribution (Satyamurti, 1967) there is no complete normal table on its development. The table by Ramaswami and Lakshman (1959) described its development up to tadpole stage only. This table in the present investigation has been prepared after induced breeding. While describing the stages help has been taken from Gosner (1960), Nieuwkoop and Faber (1967) and Agarwal and Niazi (1978). Each new significant morphological change characterizes a new development stage. The development from the time of fertilization to the time of metamophosed froglet stage has been divided into 40 distinct stages, 1 to 22 embryonic and 23 to 40 post-embryonic. As this frog species is available throughout Indian sub-continent this table can be profitably used in laboratories all over the country.

REVIEW OF LITERATURE

The knowledge of successive ontogenetic stages in anurans is considered a formost necessity, basic to training of embryologist world over. Normal tables of development have been described for a number of anuran species dividing the development into stages, each based on notable morphological change. Following are more important references on normal tables. Adler (1901) described the complete development of *Bufo vulgaris* into 15 stages. Pollister and Moore (1937) explained the development of *Rana sylvatica*, up to the development of limb bud in 25 stages. Shumway (1940) described the development of *Rana pipiens* into 25 stages up to the complete opeculum stage of the tadpoli. In 1942, he refined his earlier description with the help of histological studies. Taylor and Kollros (1946) described the post-embrynic development of *Rana pipiens* larvae from Shumway stage 25 onward and divided the larval stages into another 25 stages. Kopsch (1952) described the development of *Rana fusca*, from fertilization to metamorphosis in 30 stages. During the same year Del conte and Sirlin described the development of *Bufo arenarum* up to the operculum complete stage tadpoles, in 25 stages. Michnecwska Predygier and Pigun (1954) described the early developmental stages of *Rana temporaria*, *Rana terrestris*, *Rana esculanta* and *Bufo bufo*. Cambar and Marrot (1954) described the development of *Rana dalmatina* into 54 stages up to metamorphosis. Cambar and Gipoulous (1956) gave a chronological table of embryonic and larval development of *Bufo bufo*. In 1957 Gallien and Houillon described the development of Discoglossus pictus into 32 stages till the limb bud starts appearing. Cambar and Martin (1959) described the normal development table of Alytes obstctricans and Tahara (1959) the development stage of Rana japonica. In 1960 Gosner gave a simplified table for staging anurans embryos and larvae up to 46 stages with notes on its identification. Khan (1965) has described complete development of Bufo melanostictus into 43 stages. Many developmental tables have been provided for Xenopus laevis (Peter 1931; Gasche 1944; Bretscher 1949 and Nieuwkoop and Faber 1957), of these most exhaustive developmental table for Xenopus laevis has been provided by Nieuwkoop and Faber (1957). They have divided complete development of this species into 66 stages, based on external as well as internal anatomical structures. Since, Xenopus laevis has proved to be very favourable experimental material in many laboratories world over, these stages have proved to be of great help in performing various experiments at different stages of development. In past two decades many more developmental tables have appeared e.g. Limbaugh and Volpe (1957) on Bufo valliceps, Hing (1959) on Rana chalconata, Jorquera and Iazquierdo (1964) on Rana chilena, Sedra and Michael (1961) on Bufo regularis, Pugin and Goicoechera (1974) on Rhinoderma darwani, Jorquera and Pugin (1975) on Rana chilena and Anstis (1976) on the development of larvae of *Litona verreauxi*.

Contributions on development of frog species available in India are comparatively few. Ramaswami and Lakshman (1959) gave a normal development table of *Rana cyanophlyctis* (without description) up to embryonic (hatching) stage only, Agarwal and Niazi (1977) described the normal development of *Rana tigrina*, up to the formation of froglet in 55 stages. Roy and Khare (1978) have provided a complete normal table of *Rana limnocharis* dividing its development from fertilization to froglet stage in 32 stages. Among Indian anuran species this being first description of developmental stages formed after induced breeding.

MATERIAL AND METHODS

The frogs were induced bred (for technique see Chapter VI) and development was observed under stereoscopic binocular microscope. Fertilization was confirmed by 1st cleavage. Unfertilized and abnormal embryos were periodically removed. Cleavaging eggs were placed in crystallyzing disches, about 30 each in 200 ml of culture medium. The staging of the embryos and larvae was based on the criteria prescribed by other workers (such as Gosner 1960; Nieuwkoop and Faber, 1967; Agrawal and Niazi 1977). At each stage the embryos and larvae were fixed in Bouin's fluid.During embryonic stage, jelly was removed before fixation. At early stages the embryos were rolled on paper with fine forcep till jelly was removed, at later stages it was easily removed by forceps After hatching, the larvae were reared in stream water, to which some algae, water plants and semi boiled spinach were added as food for tadpoles at later stages the tadpoles were transferred in larger trough, partly filled with stream water and set with hydrophytes, sloping sand base on one side and stones were kept to provide amphibious environment for their metamorphosis. Diagrams were made with camera lucida and photomicrographs were taken with Ashahi Pentex set up.

OBSERVATIONS

The eggs were stripped from the females injected with pituitary extract and inseminated artificially in freshly prepared sperm suspension. The morphologcal changes were observed soon after fertilization. The fertilized eggs were transferred to stream water in a glass container (pH 5.40 - 7.50 ; conductivity, 7.5 10 x micrmhos/mm -

38.1 x 10 ppm and alkalinity 26.0 ppm to 48.0 ppm). The development was completed in 68 days at 17°C - 23°C (room temperature). It has been divided into 40 stages. Each notable morphological change, characterises a new developmental stage.

Stage 1. Age 0 hr . Length 1.5 mm . Egg spherical in shape. One cell stage before fertilization. Pigmentation darker dorsally (upper side) than vintrally (lower). The egg surrounded by thin transparent vitelline membrane.

Stage 2. Age 1 hr 15 min - 1 hr 45 min . Length 1.5 mm "One cell stage" after fertilization, spherical in shape. Dark pigmented animal pole points upwards and lightly pigmented vegetal poles downwards. The jelly capsule swells. The lightly pigmented grey cresent can be noticed at one side between the animal and vegetal hemisphere. Fig. 5.1.1

Stage 3. Age 1 hr, 45 min - 2 hr, 15 min . Length 1.5 mm "Two cell stage". A furrow originated at the animal pole developing into two equal blastomeres, formed by a meridional cleavage furrow. The grey crescent is more pronounced. Fig. 5.1.2

Fig. 5.1 : Developmental stages of _Rana cyanophlyctis_ from stage 1 to stage 15

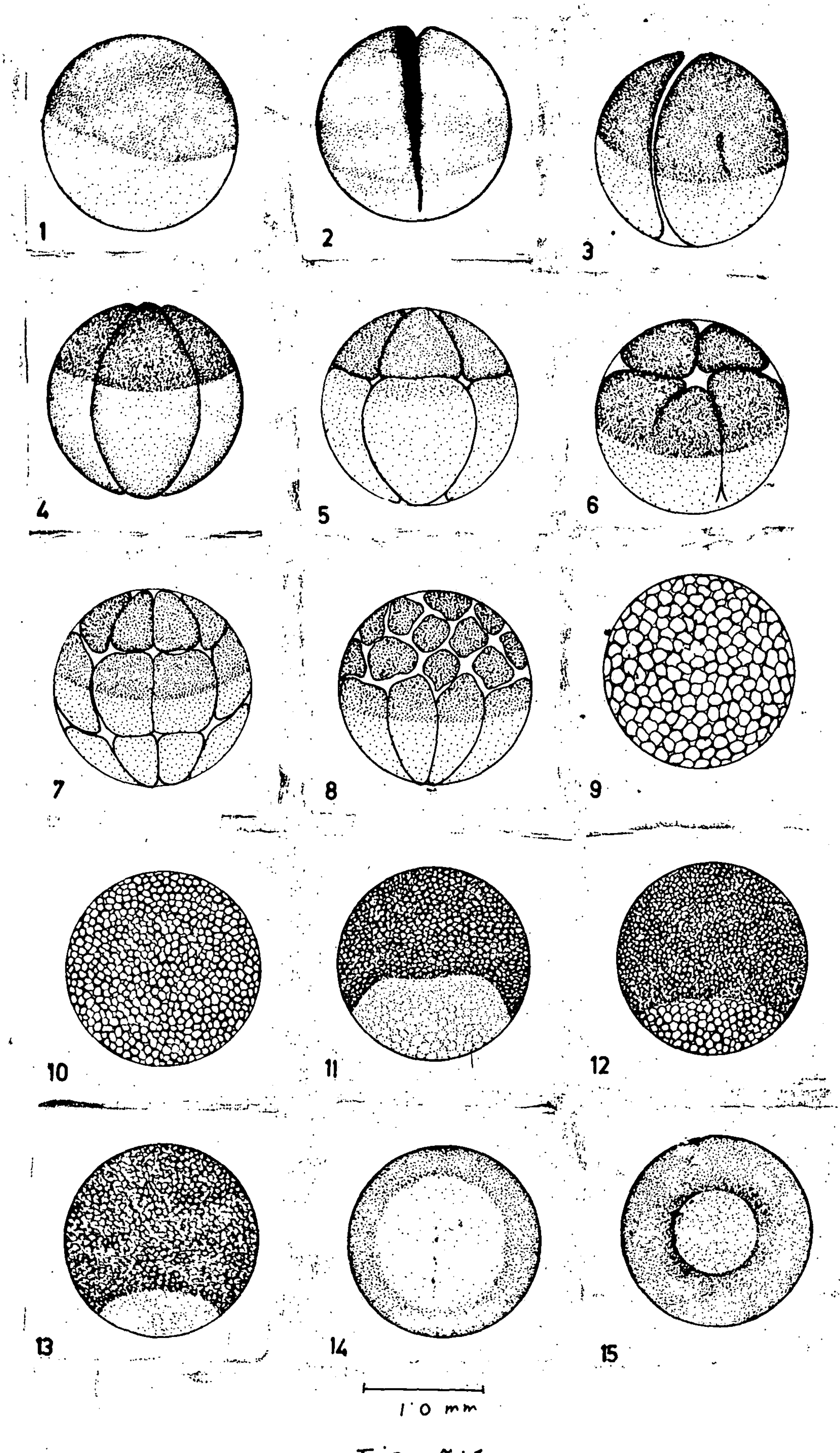

Fig. 5·1

Stage 4. Age 2 hr 15 min - 2 hr 30 min . Length 1.5 mm "Four cell stage". The second cleavage furrow extends meridionally, from animal pole to vegetal pole at right angle to the first, giving rise to four blastomeres. Fig. 5.1.3 and 5.1.4

Stage 5. Age 2 hr 30 min - 3 hr . Length 1.5 mm "Eight cell stage". The third cleavage furrow is longitudinal and slightly above equator. The four cell (micromeres) at the animal hemisphere are smaller and highly pigmented. The four larger cell (macromeres) at the vegetal pole are less pigmented. Fig. 5.1.5

Stage 6. Age 3 hr - 3 hr 15 min . Length 1.5 mm "Sixteen cell stage". The four micromeres and four macromeres divide by vertical cleavage furrows into 16 cells, 8 micromeres and 8 macromeres. Fig. 5.1.6

Stage 7. Age 3 hr 15 min - 3 hr 30 min . Length 1.5 mm "Thirty two cell stage" 32 blastomomeres are formed by the division of 8 micromeres and 8 macromeres. Except in the increase of the numbers of blastomeres, the external feature of the embryo remains same as above stage. Fig. 5.1.7

Stage 8. Age 3 hr 30 min - 4 hr 30 min . Length 1.5 mm "Sixty four cell Stage or mid cleavage stage or large cell blastula stage". The blastomeres on the embryo looks like a mulbarry. Micromeres are more in numbers than macromeres, indicating a synchronous nature of division. The cleavage furrow in the vegetal hemisphere not sharp. The distinction between the micromeres and macromeres due to gradual transition of cells from animal to vegetal pole becomes more apparent. Fig. 5.1.8

Stage 9. Age 4 hr 30 min - 8 hr . Length 1.5 mm "Medium cell blastula stage". The blastomere cells increase in number but become reduced in size. There is not much distinction between micromeres and macromeres. Fig. 5.1.9

Stage 10. Age 8 hr - 11 hr 45 min . Length 1.5 mm "Many cell or late cleavage stage" also called "Fine cell blastula stage". The cells become smaller and smaller and the surface of embryo tends to become smooth. The size of embryo gets some what increased. The distinction between micromeres and macromeres vanishes gradually. The darkly pigmented part of the animal pole enchoaches over the area of medium pigment across the equator to the vegetal hemisphere. Fig. 5.1.10

Fig. 5.12 : Developmental stages of *Rana cyanophlyctis* from stage 13 to stage 20.

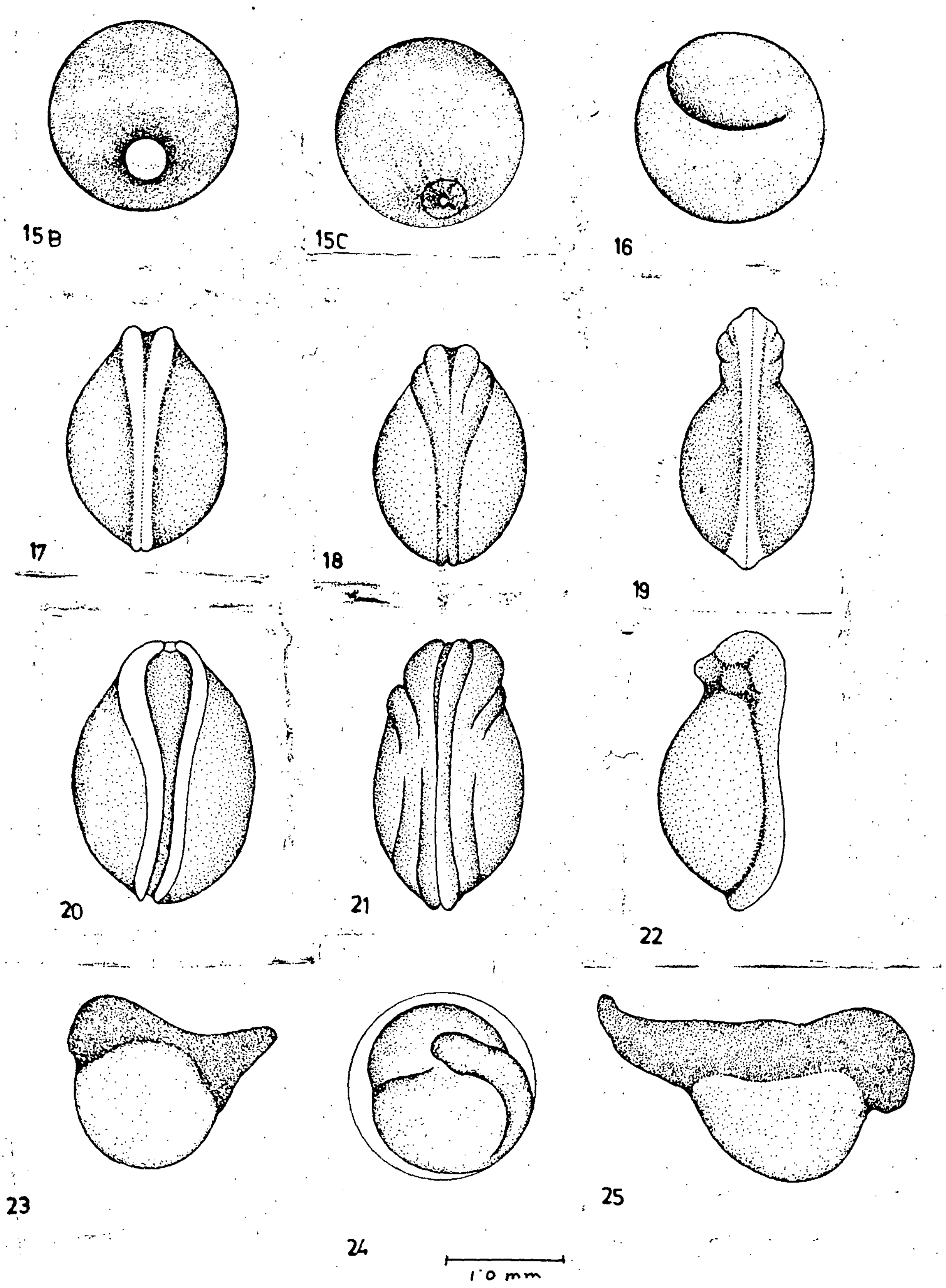

Fig. 5·2

Stage 11. Age 11 hr 45 min - 14 hr . Length 1.55 mm "Initial gastrula stage". It is marked by the beginning of invagination in the middle of the future dorsal lip of blastopore. The dorsal lip appears as crescent shape. The unpigmented zone of the vegetal hemisphere gets further reduced due to the continued migration of the pigmented micromeres towards the vegetal poles. Fig. 5.1.11

Stage 12. Age 14 hr - 24 hr 30 min . Length 1.55 mm "Blastopore or mid gastrula stage". The crescent shape, blastopore has been added by lateral lips and which is now in semicircular shape. Active gastulation is indicated by blastopore formation and continuous migration (invagination) of the blastopore cells into the interior of the embryo. The vagital hemisphere greatly reduces the exposed area of unpigmented macromeres. The semicircular blastopore yolky ultimately gets into circular stage. Fig. 5.1.12 and 5.1.13

Stage 13. Age 24 hr 30 min - 29 hr . Length 1.55 mm "Yolk plug stage". Blastopore closes ventrally. The uninvaginated macromere surrounded by the blastoporal lips protrude out a little and constitute yolk plug. Yolk plug fairly large. Fig. 5.1.14 & 5.1.15

Stage 14. Age 29 hr - 33 hr . Length 1.55 mm "Disappearing yolk plug stage". Diameter of the blastopore becomes reduced by constriction. The size of yolk plug becomes reduced though it is more surrounding through blastopore than at previous stage. Further position of the neural plate and groove become indication by dark pigmented lines extending from blastopore dorsally over. Fig. 5.2.15

Stage 15: Age 33 hr - 36 hr . Length 1.65 - 1.75 mm "Neural plate stage". Neural plate delimited embryo becomes elongated anterio posteriorly. The dorsal surface slightly flattened forming neural (medullary) plate. With high pigmentation. Fig. 5.2.16

Stage 16. Age 36 hr - 41 hr . Length 1.75 - 2.10 mm "Neural fold stage". The neural folds are prominent on the edges of the neural plate. At anterior region, the prominent neural fold extends ventrally towards caudal region to surround the small blastopore slit. Embryo looks pear shape. Fig. 5.2.17

Stage 17. Age 41 hr - 51 hr . Length 2.10 - 2.40 mm "Neural groove stage". Embryo oblong in shape. The elevated neural folds grow towards each other in anterior than in the posterior region forming a shallow groove, the neural or medullary groove. Head and trunk region can be distinguished. Lateral outline of the embryo convex. Fig. 5.2.18 and 5.2.19

Stage 18. Age 51 hr - 60 hr . Length 2.4 - 2.65 mm "Neural tube stage". Embryo length increased up to 2.65 mm. Neural folds completely fused to form the neural tube which stands out as a mid dorsal ridge. Head and trunk distinctly demarcated. Blastopore closed completely. Two faintly pigmented spots on the ventral side of the head marks the rudiments of oral sucker. Fig. 5.2.20 & 5.2.21

Stage 19. Age 60 hr - 75 hr . Length 2.65 - 2.9 mm "Tail and stage". Tail bud prominent at the posterior end of the embryo. The optic bulges on the head are some what posteriorly placed. The bulges of gill plates prominent on each side. Anterior to the optic bulge a pigmented spot marks the area of the future olfactory pit. Stomodaeal groove present in the form of longitudinal dipression, in the mid ventral region of the head laterally behind this groove a pair of oral sucker appear as a slightly pigmented conical protuberance. Fig. 5.2.22 & 5.2.23

Fig. 5.13 : Developmental stages of <u>Rana cyanophlyctis</u> from stage 21 to stage 25

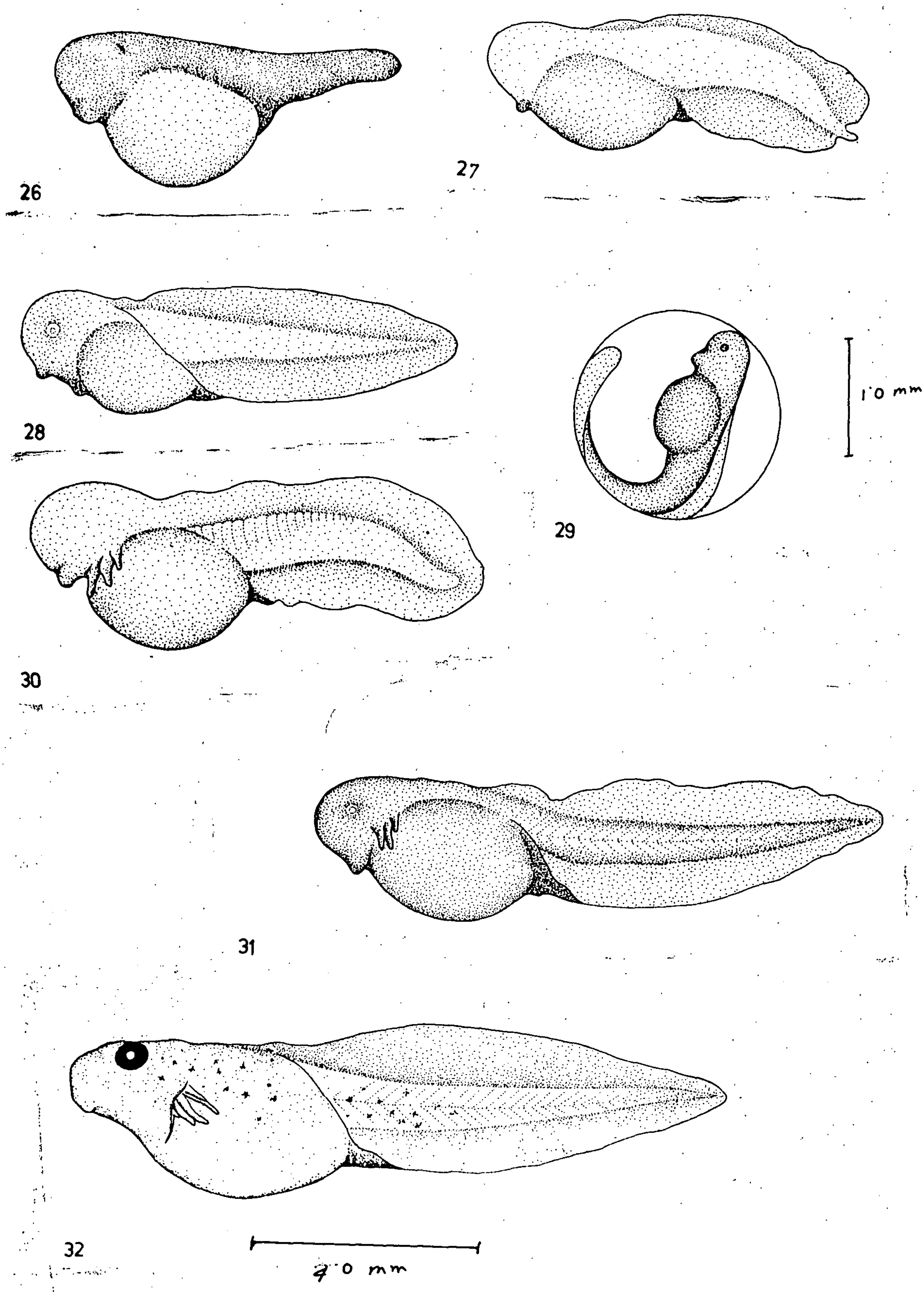

Fig: 5·3

Stage 20. Age 75 hr - 80 hr . Length 2.9 - 3.4 mm "Muscular response stage". Distinct eye. Tail bud enlarges. Small pigmented depressions in front of each optic bulges mark the position of Olfactory pit. Oral sucker prominent. Stomodeal groove deeper and anterior to small pits. Myotomes are visible. Fig. 5.2.24 & 5.2.25

Stage 21. Age 80 hr - 90 hr . Length 3.4 - 4.5 mm "Heart beat stage". Tail fin formation clearly distinct. Dorsal and ventral fins prominent. Myotomes distinct and extends approximately half the length of tail. Oral sucker becomes more prominent. The head and trunk clearly separated. Fig. 5.3.26 and 5.3.27

Stage 22. Age 90 hr - 110 hr . Length 4.5 - 5.3 mm "Gill circulation stage". Tail bud elongates into distinct tail. The onterior part of the stomodoeal groove depends. Distinction between jaw and gill area well marked. Oral sucker enlarges. Eye spot clear. Tail fin are outer transparent and inner translucent. Muscles with hands can now be observed in the tail fins. Broadest fin area behind middle of the candal half. The embryo hatches at the stage. Fig. 5.3.28 and 5.3.29.

Stage 23. Age 110 hr - 130 hr . Length 5.3 - 3.8 mm "Mouth open stage". Mouth distinct open and consists of whitish break. Three pairs of external gills well marked with fingers like filaments. Pigments visible at head trunk and tail. Cornea transparent and lens visible as white spot. A rudiment of skin fold visible at the base of the external gills. Larvae show swimming. Occasional jumps and movements by muscle contraction, in culture media. Fig. 5.3.30

Stage 24. Age 130 hr - 155 hr . Length 6.8 - 8.0 mm "Tail fin circulation stage". The head, eye, ears, mouth and gills prominent. The epidermis becomes transparent. Mouth opening surrounded by upper and lower labial fringes. Blackish brown kerotodonts and oral papillae visible in the mouth. Upper folds develops over the gills at each side. Tail has broad tail fins and tail myotomes can be seen. Fig. 5.3.31

Stage 25. Age 155 hr - 210 hr . Length 8.0 - 8.5 mm "Opercular fold stage". Gill filaments thin and transparent. Larvel mouth surrounded by pre and post oral labial fringes. Larvel beaks has black keratinized sheath with cutting edges.

Fig. 5.4 a Developmental stages of Rana cyanophlyctis from stage 25 to stage 31

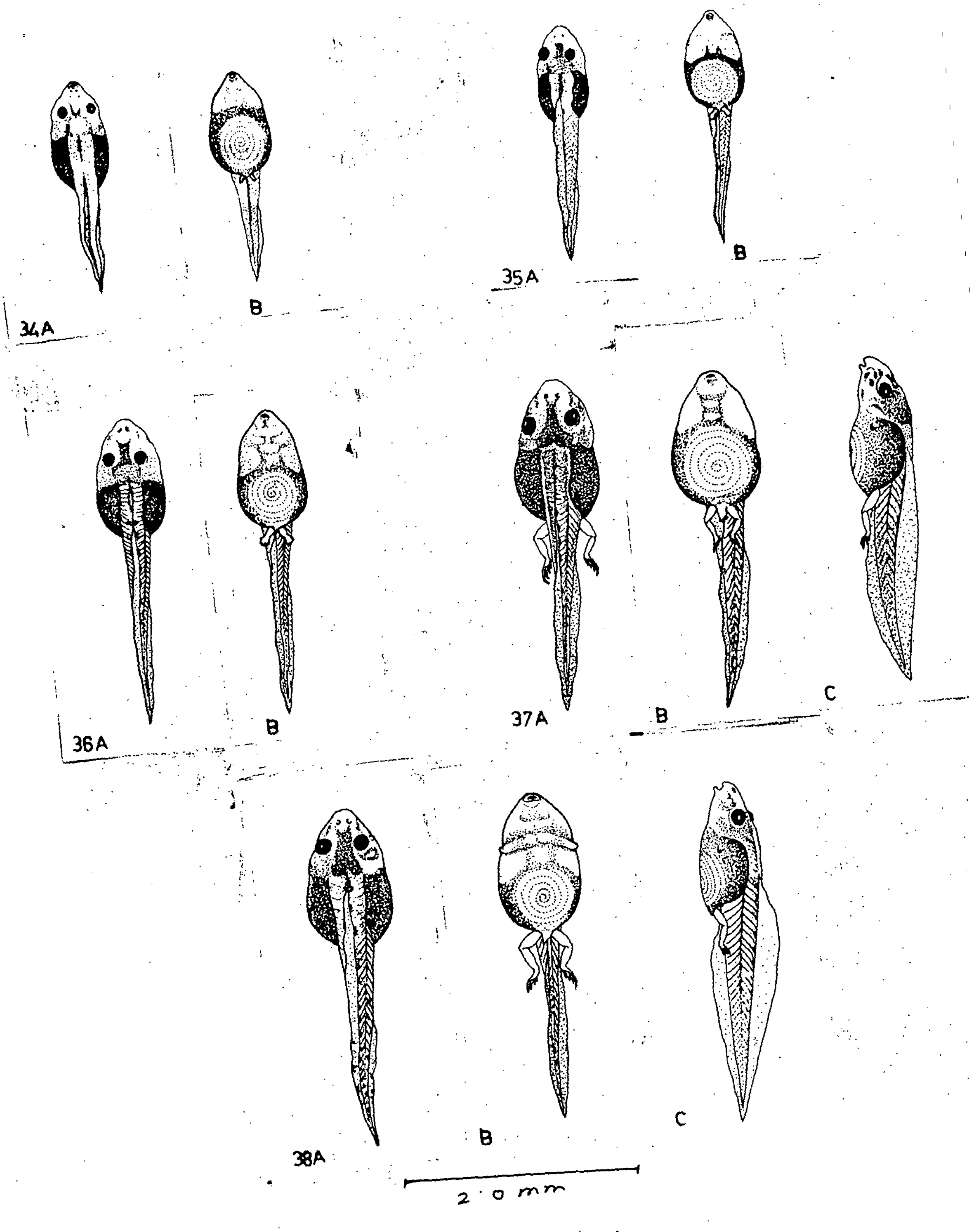

Fig. 5.4

Transparency of the skin increases. Numerous melanophores at dorsal or lateral side of the animal. The operculum grows positively. Fig. 5.3.32

Stage 26. Age 210 hr - 260 hr . Length 8.5 - 9.2 mm "Right side operculum closed stage". The operculum fused with anterior belly skin on the right side covering the external gills. Gills at the left side still visible although smaller in size. Oral sucker reduced. Mouth structure more clear and shows dark brown horny teeth on pre and post oral labial fringes. Fig. 5.3.33

Stage 27. Age 260 hr - 285 hr . Length 9.2 - 10.5 mm "Complete operculum stage". The operculum fused with the skin of anterio-ventral billey on both sides. In small ventro lateral area, however, a portion of gills still visible, on the left side through a ventro lateral edges of operculum. Oral suckers much reduced. Two coils of intestine visible through ventral skin. Todpole start feeding on filaments algae. Demarcation between head and trunk disappear, thus embryo has only trunk and tail. Eyes well developed and lens with retina. Fig. 5.3.34

Stage 28. Age 285 hr - 384 hr . Length 10.5 - 18.5 mm "Hind limb as long as wide stage". Hind limb buds as long as wide and appear at the groove between the base of the tail and belly. Elevation of oral sucker disappears. Eye prominent lateral line system present. Pigmentation distributed throughout the body surface of the tadpole. Intestine has 4 - 4½ coils. Fig. 5.4.35

Stage 29. Age 384 hr - 576 hr . Length 20.0 - 26.5 mm "Foot paddle stage". Distal end of the hind limb bud becomes flattened medio-laterally to form a foot paddle. Hind limb approximately twice long as wide and extends beyond the cloacal opening. The ventral depression in the middle of hind limb delimits the proximal segments or thigh and it indicates the position of the knee band. Intestine shows 6 - 6½ coils. Fig. 5.4.36

Stage 30. Age 576 hr - 912 hr . Length 28 - 34.5 mm "Fourth interdigital indentation". The morgun of the foot paddle indented between all four toils, which are now separated from each other Mclonophons patches visible on thigh, shank ankle and foot region. The number of horn teeth

Fig. 5.5 : Developmental stages of *Rana cyanophlyctis* from stage 32 to stage 33

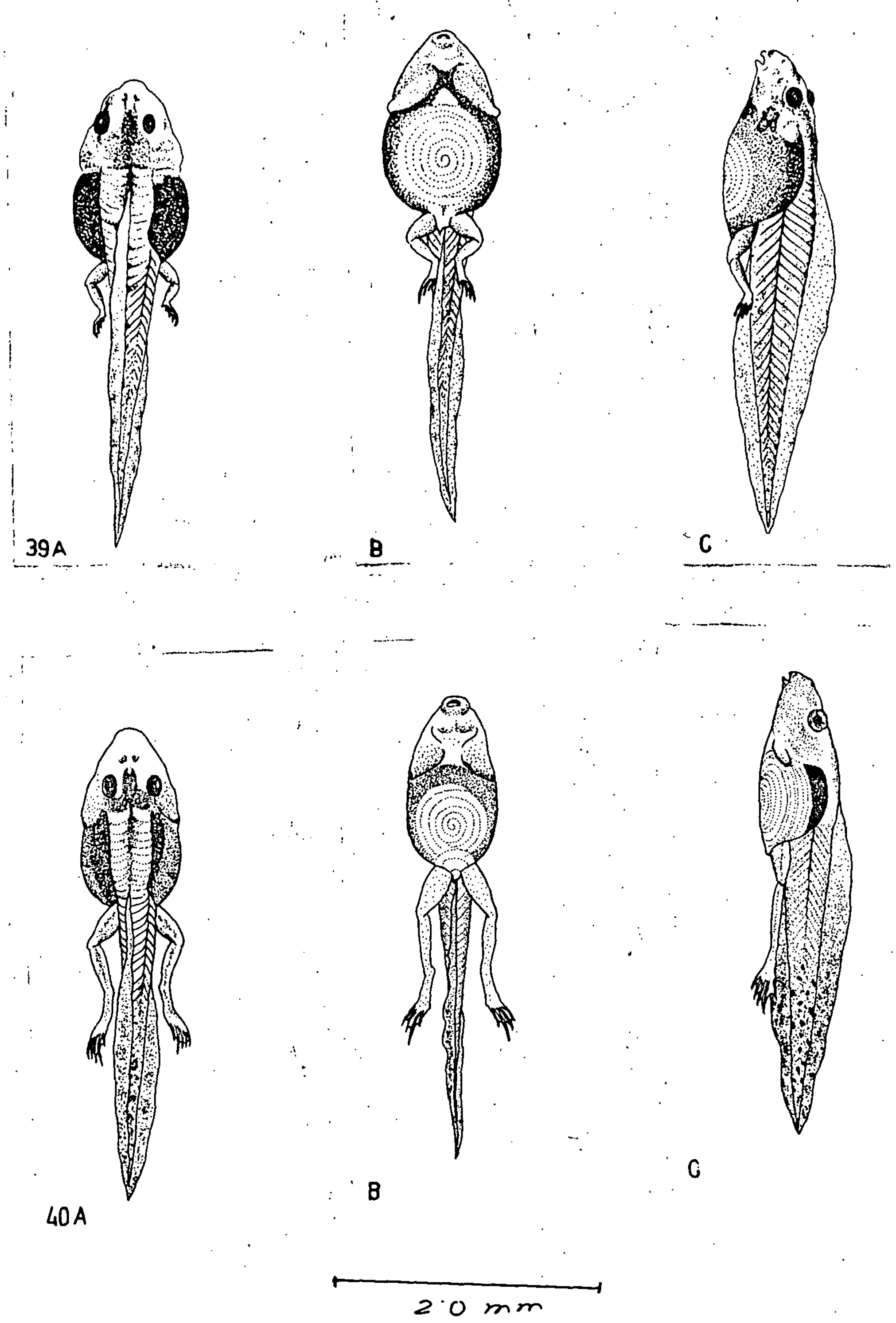

Fig. 6.5

increases on pre and post lips. The margin of the 5th toe web directed towards the tip of third toe. The distinct half of the hind limb extends beyond the opening of the cloacal tail piece. Fig. 5.4.37

Stage 31. Age 912 hr - 1008 hr . Length 34.5 - 44.0 mm "Demarcation between tarsal and metatarsal region". Tarsal and metatarsal region can be recognized separately. An angle of 90° can be noted between the thighs. Base of the cloacal piece narrower than previous stages. Webs well developed and individual pharengeals recognizable externally. Two ankles almost parallel to each other. Fig. 5.4.38

Stage 32. Age 1008 hr - 1200 hr . Length 45.0 - 58.0 mm "Phallangeal differentiation". Phallanges phallangeal out growths distinct and can be made out externally. The nasolaerineal duct visible as a white line between the nostril and eye. Bases of the two thighs have come closer to each other madially reducing the proximal part of the cloacal tail piece to a narrow strip. Thigh makes acute angle with the shank. Fig. 5.5.39

Stage 33. Age 1200 hr - 1392 hr . Length 58.6 - 63.5 mm "Pelvic Symphysis". Bases of the two thighs almost fused with each other, madially indicating the formation of pelvic symphysis, however, small distal part of the cloacal tail piece remains dorsal to thigs. Fig. 5.5.40

Stage 34. Age 1392 hr - 1464 hr. Length 60.0- 65.0 mm "Fore limb visible". The fore limb visible through the transparent skin at the ventral side. Elbows found jutting against the body wall laterally. Cloacal piece disappearing. Dissolution of the skin below the left elbow begins and forms a window for subsequent emergence of the left fore limb. Fig. 5.6.40

Stage 35. Age 1464 hr - 1476 hr . Length 65.0 - 63.0 mm "One fore limb stage". Only one fore limb emerges. The left fore limb emerged first in the three cases observed. It emerges through a window formed by the enlargement of the spiracular opening. Simultaneously fin resorption begings. Intestine length reduces. Fig. 5.6.41

Stage 36. Age 1476 hr - 1482 hr . Length approximately 60.0 mm "Two forelimb stage". Both fore limbs have now

Fig. 5.6 : Developmental stages of <u>Rana cyanophlyctis</u> from stage 34 to stage 40

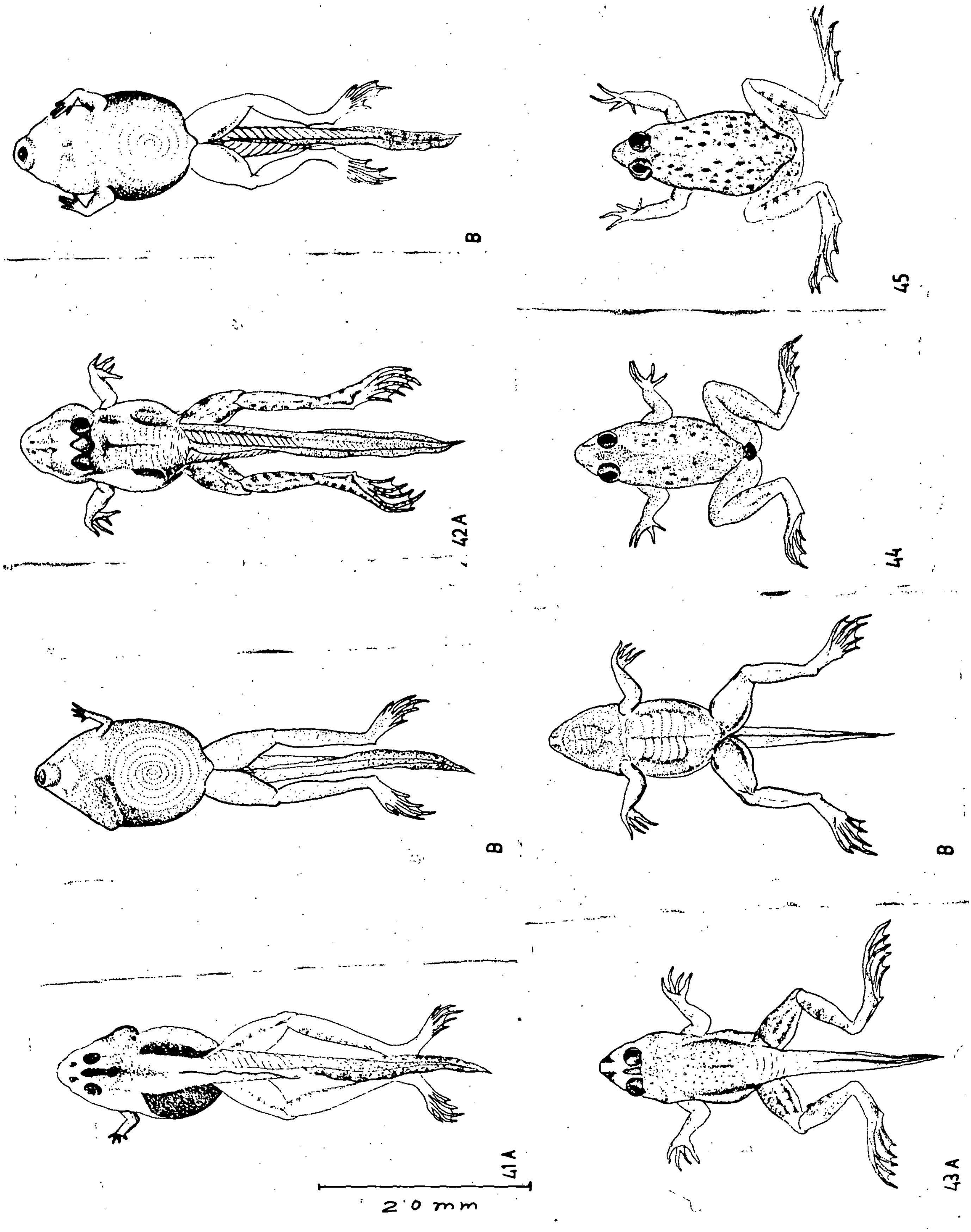

Fig. 5.6

emerged. The right fon limb emerges from the window formed by the dissolution of the skin below its elbow. Head broader than trunk. Tadpole size reduces approximately 3 mm. Intestine length further reduces and is smaller than stage 35. Rows of teeth and horny beak still present. Fig. 5.6.41

Stage 37. Age 1512 hr . Length 54.0 - 50.0 mm "Mouth gape widening. Labial fringes disappeared and horny teeth and beaks have been shed. Mouth gape widens. Small tongue developes. Nictitating membrane perceptible. Trunk gets narrower than the head. Adult types pigmented patches appear on the body and limbs. Tail and the size of the animal further gets reducing. Fig. 5.6.42

Stage 38. Age 1548 hr . Length 26.0 - 31.0 mm "Reduced tail stage". Tail resorbed and reduced to about ¾ of its length. Dorsal fin either greatly reduced or absent. Mouth gape widens. Snout assumes adult form. Eyes prominent and buldging. Fig. 5.6.43

Stage 39. Age 1584 hr . Length 22.0 - 26.0 mm "Tail stub stage". Tail reduced to a stub corner of the mouth reaches the level of the posterior margin of the eye. General body shape like that of adult. At this stage froglets are observed often sitting on stones close to roater. Fig. 5.6.44

Stage 40. Age 1632 hr . Length 22.0 - 18.0 mm "Froglet stage". Fully metamorphosed froglets. Tail completely disappears. Patches of pigments are seen on the body surface like those of adults. Metamorphosed froglets shows all anuran characters and are miniature image of its species, without any external distinguishable sexual characters. Fig. 5.6.45.

DISCUSSION

General:

McCann (1933) and Ramaswami and Lakshman (1959) mentioned that Rana cyanophlyctis can breed throughout the year, at suitable localities. During present study, Rana cyanophlyctis was found active and available all around year at Shillong (1425 m.a.s.l.) as well as at Gauhati (49.4 m.a.s.l.). On the basis of the availability of large number of females with uterine eggs. Ramaswami and Lakshman (1959) reported that Rana cyanophlyctis breeds actively during early part of August. Based on the relative conditions and number of eggs in the ovary during different months, its active breeding appears to be during monsoon season but induced breeding can be successfully achieved in this frog almost every month from March till October (see Chapter VII).

Duration of development:

The present developmental table of *Rana cyanophlyctis* has been prepared in the months of June, July and August at room temperature from 17°C - 22°C. Its development from fertilization to froglet stage was completed in 1632 hours. The chronological timing of development are following:

Embryonic - 8-12 hr; gastrulation 14-24 hr 30 min; neurulation 51-60 hr; hatching 90-110 hr.

Post-embryonic - Formation of external gells: 110-140 hr; Operculum fold stage: 155-210 hr; hind limb stage: 260-285 hr; five toes stage: 576-912 hr; hind limb enlarged stage/phallangeal differentiation stage: 1008-1200 hr; large size active feeding tadpole: 1392-1464 hr; fore limb stage: 1482 hr; and total disappearance of tail and metamorphosis to froglet: 68 days i.e. 1632 hr.

So far as hatching time is concerned Ramaswami and Lakshman (1959) described hatching of *Rana cyanophlyctis* at 116 hr of fertilization at 18°C - 20°C. Agarwal and Niazi (1977) reported 17½ hr for *Rana tigrina* at 28°C - 32°C. Roy and Khare (1978) reported 120 hr as hatching time for *Rana limnocharis* at 17 °C - 21.6°C. The same species from Singapore has been reported to hatch 24 hr after fertilization at temperature 27°C - 29°C. Roy and Khare (1978) discussed that the temperature was a plausible factor for the difference in the hatching period of the frog at Shillong and at Singapore. They further discussed that high temperature (26°C - 30°C) at which Agarwal and Niazi (1977) studied the development of *Rana tigrina* is accountable for its faster developmental rate during embryonic stages and slower developmental rate at post embryonic stages. The hatching period of *Rana cyanophlyctis* at Shillong at 17°C to 21°C in the present investigation and at Bangalore at 18°C - 20°C as reported by Ramaswami and Lakshman (1959) were noted to be almost same.

Although in our laboratory, at Shillong, the hatching time of *Rana cyanophlyctis* (in the present investigation) and *Rana limnocharis* (Roy and Khare 1978) were observed to be almost same, the duration the post-hatching stages and metamophosis were found to vary greatly. *Rana limnocharis* (Roy and Khare 1978) completes its development and metamophosis on its 33rd to 36th day of fertilization but Rana cyanophlyctis requires a prolonged period of approximately 68 days. Earlier Taylor and Kallors reported 90 days for the completion of metamorphosis in Rana pipiens , Nieuwkoop and Faber (1967) reported 58 days for Xenopus laevis and Agarwal and Niazi (1977) 52 days for Rana tigrina. Though development appears temperature dependent inherent genetic factors may also be operating in a species.

The growth though slow during embryogenesis becomes faster after hatching particularly after stage 27 (hind limb bud stage). At this stage the development of operculum gets complete and larvae behaves as a ravenous vegetative feeder. Growth continues up to stage 34. The metamorphic degrowth is observed subsequently and continues up till froglet is formed on the 68th day.

Every marked ontogenic change is taken as a new developmental event of Rana cyanophlyctis forming the basis for a new stage. Although the criteria followed by different authors are similar, yet there have been lot of variation in the numbering of stages of different anurans. Shumway's (1940) developmental table for embryonic and Taylor and Kollras's (1946) developmental table for the larval period of Rana pipiens have been invariably used for reference by many investigators and laboratory manuals. (Humburger, 1960; Rugh, 1962; Witschi, 1953,1956,1962).

Gosner (1960) proposed criteria for the staging of frog embryos. Though these criteria are immensly helpful but sometimes it becomes difficult to rigidly stick to any one of these due to differential developmental pattern of different species and due to inherent genetic variability. Jorquera, Pugin and Goicoechea (1974) observed that embryonic structures, stages and developmental time varied in the different population of *Rhionoderma darwini* probably due to their adaptibility at different requirements and conditions of the sites. Among all normal tables available the developmental table of *Xenopus laevis* described by Nieuwkoop and Faber (1967) is the most exhaustive. They have followed external and internal morphological changes as a criteria for staging, specially because *Xenopus laevis* has been found to be one of the most suitable material for the developmental studies in many western laboratories. In the present investigation help has been taken from Gosner (1960), Nieuwkoop and Faber (1967), Agarwal and Niazi (1977) and Roy and Khare (1978). The development has been divided into 22 embryonic and 18 post embryonic stages.

In the last, a word can be added on its habit. It is an aquatic frog. Carr (1940) commented that for biological studies aquatic frogs have proved to be ideal species as they remain relaxed when held in hand and hangs their limbs, rarely resorting to vigorous kick and contusive behaviour, unlike terrestial anurans. Rana cyanophlyctis being of handy size, aquatic in nature, abundantly available throughout the year in the Indian subcontinent, can be used as a convenient class room material for development studies under Indian conditions. It does not undergo hibernation. It has prolonged breeding period. Attempts can be made to use it as aquarium frog in the laboratory.

SUMMARY

The present chapter deals the description of normal table of Rana cyanophlyctis Schneider. The development has been studied after induced breeding. The rearing of embryos and larvae has been carried on in the laboratory in the pond water (Temp. 17°C - 21°C ; pH 6.46 ; conductivity 19.2 x 10 micrhomos/mm ; oxygen 8.13 ppm). Hatching occurs on 4th/5th day and metamorphosis into froglet stage is completed on 68th day. The staging of the embryos has been based on major changes in the external morphological feature. The entire development from fertilized egg up till metamorphosis has been divided into 40 stages; fertilization stages 1-2 ; cleavage stages 3-9 ; gastrulation stages 10-12 ; neurulation stages 12-18 ; organogensis stages 18-24 and post embryonic development stages 24-40. Rana cyanophlyctis is a prolonged breeder and can be induced to breed throughout the year under ideal conditions.

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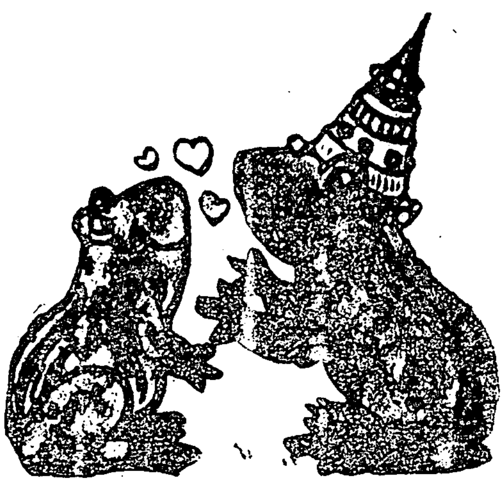
CHAPTER—6

Experimental Breeding



INTRODUCTION

In recent years induced breeding has been profitably used for aquaculture programmes. But its standardization is an important prerequisite for any species. The role of pituitary in the reproduction of vertebrates was first understaad from the experiments of Aschheium and Zordek (1927), when they investigated that pituitary implants accelerate sexual development in mice (for details see Allen, 1939 and Pickford and Atz, 1957). In the year 1929, Wolf on *Rana pipiens* and Houssey *et al*. (1929) on *Bufo marinus*, performed pioneering experiments demonstrating the induced ovulation in anurans by injecting or implanting homoplastic pituitary glands. Subsequently, many biologists such as, Adams (1931); Wills *et al*. (1933); Shapiro (1936); Bellerby (1933); Rugh (1934, 1935a, b, 1939, 1962); Creaser and Gorbman (1935); Gallian (1937); Ramaswamy and Lakshman (1958, 1959); Wright (1945, 1950); Wright and Hisaw (1946); Wright and Flathers (1961); Nieuwkoop and Faber (1967); Hock and Wen (1970); confirmed ovulation in anurans by pituitary hormones and steriod injections. Based on this principle certain biological companies in U.S.A. (such as Carolina, Burlington NC and Turtox Chicago) supply pituitary kits for induced breeding in frogs.

While working on *Rana tigrina*, *Rana hexadectyla* and *Rana cyanophlyctis* Ramaswamy and Lakshman (1959) expressed concern that in India techniques of induced breeding are not adequately developed and prescribed a more favourable technique using the homoplastic as well as fish pituitaries, in combination with threshold dosage of mamalian hormones for induced ovulation in these species. But even now induced breeding is not conveniently practiced in many Indian laboratories.

Rugh (1948) proposed that the pituitaries can be stored in absolute ethyl alcohol without losing their potency. Use of preserve pituitaries is now a routine for induced breeding in fishes (see Jhingran, 1975). Khare, Roy and Kumar (1981) demonstrated that frog pituitaries preserved in absolute ethyl alcohol retain their potency for several years and can be used profitably for induced breeding in frogs. The procedure followed in the present investigation for *Rana cyanophlyctis* is based on the combination of techniques prescribed by Rugh, (1934) Osche, (1968) and Jhingran (1975). According to this technique, a stock of anterior pituitaries of male and female frogs preferably of same species preserved in absolute ethyl alcohol is built up and a measured quantity of these pituitaries is used at the time of induced breeding experiment. *Rana cyanophlyctis* has a prolonged breeding period - March to October at Shillong and still longer at Gauhati. This chapter describes how these preserved pituitaries are successfully used for induced breeding of this species under laboratory conditions.

Induced breeding technique can be used for experimental breeding of the animal on large scale. As gonado somatic index and fecundity may be helpful in experimental breeding programmes they have also been included in the present chapter.

REVIEW OF LITERATURE

The concept of the use of pituitary injection for successful spawning in frogs came up in 1929 through the work of Wolf on Rana pipiens and Houssay et al. on Bufo marinus. The following year 1930, Houssay demonstrated spawning by pituitaries in fishes also. Wolf (1929) observed that continuous transplantation of a pituitary for 3 - 4 successive days in female Rana pipiens either lead to ovulation or gorging of egg in uterus. He also experimented and found that the transplantation of brain or neural tissue had no effect on gonads or spawning. Wills et al. (1933) observed that toad can be induced ovulated by fish pituitaries. Rugh (1934, 1935) demonstrated that injection of homoplastic pituitary glads directly in the abdominal cavity brought about amplexus and ovulation. The egg thus obtained were artificially inseminated. With the help of Homoplastic and Heteroplastic pituitaries, Rugh (1935) successfully induced ovulation in Rana clamitans, Rana catesbeina, Rana palustris and Bufo floweri during different months and noted that the dosage of pituitaries varied during different months. He also noted that the pituitary of Bufo floweri was smallest in size and least potent for other species and that of Rana catesbeiana was largest and strongly effective in other species. He further demonstrated that extract of mamalian anterior pituitary (sheep gland and antuirian - 5 from urine of pregnant individuals are effective in all species of frogs. Rugh (1934) used meshed pituitary extract in distilled water or 10% ethyl alcohol for induction of ovulation in anurans, and observed that homogenate enhanced pituitary action by 300-400%. He observed that hypophysectomization in frog showed of gonads but on injecting its own pituitary later enhanced some gonadal maturation and ovulation, While concluding he also mentioned that the degree to which ovaries are emptied depends upon the temperature. Subsequently he investigated the breeding behaviour of different species of frogs found in North America, and stated that anuran eggs and larvae can be obtained in all months of a year. Rugh (1935) further observed that inter and intra-specific amplexes can be achieved in frogs, but not among frogs and toads. He believed that the warts and poisonous skin of toad had been the plausible reason for the failure of amplexes. Rugh in the year 1939 described in detail the technique of obtaining anuran eggs by anterior petuitary during breeding period of frogs(for details of technique see Rugh, 1962). Landgrebi and Purser (1941) described the technique of breeding Xenopus in laboratory. In 1942, Robinson and Hill made some modification in the injection technique proposed by Rugh (1934), for successful induced ovulation of Rana pipiens in the laboratory conditions. Adams and Granger (1938) showed that Triturus viridescens pituitary induces ovulation in Rana pipiens and felt that there was no zoological specificity of gonads stimulating hormones.

Creaser and Gorbman (1939) recorded that fish pituitaries are capable of spawning prawn. Further, Wills, Riley and Stubb (1933); Stroganor and Alpatov (1951), and Picford and Atz (1957) and Chaudhuri (1960, 1963) have also reported that spawning in fishes can be induced by frog pituitaries. In the year 1942, Creaser achieved ovulation in Rana pipiens by bird pituitary preparation. On placing the ovaries "in vitro" in the cultural medium having pituitary hormones. Wrights (1945) demonstrated growth and maturation of ova. In the year 1950, he demonstrated that the length of the exposure of ovaries in cultural medium having pituitary hormones leads to "in vitro" ovulation. Barr and Hobson (1967) regulated dosage of the gonodotrophin injection in Xenopus laevis so as to obtain desirable number of egg. They also formulated a method for the estimation of numbers of egg laid by any species. Alonso-Bedata and Serrano (1970) described a technique for experimental ovulation and fertilization in Rana ridibunda and noted that keeping female of the species at room temperature for larger period deteriorates the ovaries and ovulation capabilities. Hook and Wen (1970) demonstrated artificial breeding in Rana limnochasis following Rugh's technique and described its early development up to tadpole stages. Though there is no zoological specificity, earlier worker, Barth (1933); Creaser and Grobman (1935); Rugh (1933, 1935) mentioned that sheep or other cattle pituitaries extract and/or pregnant mare serum and/or human pregnancy urine and chorionic gonodotropin do not induce ovulation in mature Rana pipiens. But subsequently many workers have successfully induced ovulation using hetroplastic pituitaries including mammalian pituitaries and various gonodotropic steriods, such as Rugh (1935) in Bufo floweri and Rana catesbeiana; Creaser and Grobman (1935) in Hyla aurea; Ballerby (1933) in Cunnigham and Smart (1934) and Gallian (1937) in Rana temporaria and Shapino (1933), Shapiro and Zwarenstein (1939) in Xenopus laevis. Wright and Hisaw (1946) in ovulating Rana pipiens with a mixture made up of mammalian pituitary and gonodotropin steriods. In the year 1961 Wright and Fleather observed that injection of homoplastic anterior pituitary gland in combination with progesterone compels complete ovulation in Rana pipiens.

Contribution on induced breeding on frog species available in India are relatively few. Ramaswamy and Lakshman (1958, 1959) presented injection of homoplastic pituitaries in combination with threshold mammalian hormones for successful induced breeding in Rana tigrina, Rana hexadactyla and Rana cyanophlyctis. They also noted that pituitary collected from females having regressed ovaries showed indifferent result and the frog freshly collected give better result than those stored for 8 to 10 days. Further they mentioned that by altering the dosage the ovulation could also be achieved during winter month in Rana cyanophlyctis as gravid females could be procured throughout the year.

Gangadhara and Ramiah (1968) observed that Hypophysectomy and ovariectomy in Rana cyanophlyctis resulted in the atrophy of the oviduct and decrease in alkaline phosphate activities. Methallibure a non sterioid pituitary inhibitor reduced the oviducal alkaline phosphate activity. Further, starvation destroyed the mature follicle in the ovary and caused depletion of ovarian cholestrol and oviducal alkaline phosphate activity. Hence they concluded that reproduction in female skipper frog is under hormonal and nutritional control as in mammals. Kasinath and Basu (1977) investigated that different dosage of steroid had significant role on spermatogensis of the frog Rana hexadactyla.

Gopalkrishnan and Rajasekharsetty (1977) observed that Rana cyanophlyctis is a unique frog which maintains the gravity of ovary round the year. However, its active breeding season is recorded to be a prolonged one extending from July till the end of September in South India. Further they recorded that the ovary of the skipper frog shows two distinct phases. No. (i) ovarian phase during which ovary releases gravid eggs (ovulation). No.(ii) oviductal phase during which the released egg passes through the oviduct causing spawning. They also noted that pituitary homogenate induces spawning better than steroids. While the steroids acts as an effective agent for oocyte maturation (Roy, 1979) induced ovulated Rana limnocharis at Shillong under laboratory conditions using homoplastic and heteroplastic pituitaries homogenates and observed that large number of viable eggs can be obtained from the female frogs during the month of April and May, soon after the termination of it hybernating phase. During later months the frogs act indifferently due to spent ovaries. Hence she has recommended April and May for successful induce ovulation in Rana limnochanis of Shillong.

The dosage calculation and preservation of the pituitaries have been attempted by the biologists world over. Strogonov and Alpatov (1951) proposed frog unit as the unit quantity of gonadotropin that would bring about appearance of sperm in cloaca of 50% of male frogs and toads and/or that exhibit mature spermatozoa in their spermduct. However, this technique was not found suitable due to indifferent behaviour in anuran population during different months and from different ecological conditions. Jhingran (1975) mentioned that this has been the reason for not deriving any chemical standardization of pituitary gonadotropin which insures successful spawning in animals.

Alikunhi et al. (1960); Choudhuri (1960, 1963) described preservations of fish pituitary in alcohol and acetone and observed that such pituitary retain its potency hence can be conveniently used later when and where required. Ibrahim and Choudhuri (1966) devised a technique for preservation of pituitary extract in glycerine and there by providing radymade suspension for induce ovulation in animals. The technique, however, was found to be not so effective as its potency deminishes after the storage of 9 to 61 days (see Jhingran, 1975).

Gonado somatic index

Body length and its relationship with gonadial condition/weight have little been worked out amongst anuran. Liu (1950) estimated the number of egg masses and clutches size in Amolops chunganesis per ovulation per female and noted that an average female of 54.0 mm lays an approximately 2180 ova. Among temperate Ranids, Terentjev (1960) developed relationship between clutch size and the size of female and noted a linear relationship that can be expressed by a linear expression :

$$\log F = -1.7428 + 2.1670 \log L$$

where F = Fecundity, L = SV length of female in cm

Inger and Greenberg (1966) and Inger and Bacon (1968) derived relationship between body length and clutches sizes among temperate anuran and reptiles and noted linear expressions, identical to Terentjev's (1960) emperical formula. Inger and Bacon (1968) observed that in sararid forest,rain frogs breed throughout year and show similar annual seasonal and behavioural pattern throughout the year. Moreover the changes in their seasonal pattern can be known by Kruskal Wallis analysis (Seigel, 1956). Schrocder (1974) derived correlations of weight and size of tistis with the body size of male Rana catesbeiana, throughout its reproductive cycle, to find out the period for its active breeding. Koskala and Pasanen (1975) derived correlation coefficient and regression equations in <u>Rana temporaria</u> among following stuctures:

Body length and weight of ovary

$$Y = 0.01766\ X + 1.28563$$

$$(P\ \ 0.001\ ;\ N = 18)$$

Body length and weight of oviduct

$$Y = 0.02954\ X + 0.64478$$

$$(r = 0.912;\ P\ 0.001; N=29)$$

Body length and numbers of egg

$$Y = 33.394\ X - 1480.498$$

He noted that volume of spawn size and numbers of eggs are dependable on the size of female and observed that female of SVL 78.0 mm would produce 13.5 - 14.0 gm of spawn comprising about 1360 eggs of size 2.01 mm containing about 1.0 mg of magnesium ; 0.2 mg of zinc and 0.04 gm of copper.

6.3 MATERIAL AND METHODS

Collection of frog:

The specimens of Rana cyanophlyctis collected both at Gauhati and Shillong were used for induced breeding experiments. It was easier to get mature frog more abundantly at Gauhati than at Shillong (see Chapter III and Fig. 3.3). The frogs from Gauhati were transported to the laboratory in 5 and 10 litre plastic jar half-filled with pond water and covered with perferated lid or cheese cloth. It was observed that over crowing, more than 15 frogs in small and more than 30 in large container, resulted in some mortality. The pond water was replenished thrice during 101 km journey from Gauhati to Shillong, by draining out about 2/3 of water at a time.

At the laboratory they were maintained in large glass aquaria (13" x 18" x 18") covered with win mesh lid (Fig. 6.4). The aquaria were filled up to 6" pond water and were set with steep sand base at one side, and stones to maintain amphibious environment. Algae and aquatic plants were placed in the aquarium. Insects and earthworms were given to the frogs as food. Diseased frog, specially those with red leg disease (Mohanty-Hymedi, 1974), were removed periodically, as soon as such symptom was first observed. The water of the aquarium was replenished at weekly intervals and temperature was maintained at approximately 20°C by electric bulb.

Removal of pituitaries:

The frogs were pithed, and sometimes just dead or dying frogs were also used as pituitary donors as they also yielded good result. The pituitaries were dissected out as follows:

Head with upper jaws were cut transversely behind the angle of jaw and placed upside-down, in amphibian ringer solution, if experiments are to be performed with fresh pituitaries, otherwise in absolute ethyl alcohol. The parasphenoid bones were cleared and with a tip of fine scissors inserted in foramen magnum, the parasphenoid were cut longitudinally along its lateral edges and deflected gently, taking care every time not to injure underlying organs. The pituitary is located behind optic chiasma, as a transverse kidney shaped pinkish structure surrounded by some endolymphatic tissue (Fig.6.5). The pituitaries were carefully removed and placed in amphibian ringer solution or absolute ethyl alcohol, as the case may be.

Fig. 6.4 : Maintenance of frog in the laboratory condition (Aquarium) before experimental breeding.

6.5 : Position of pituitary.

Fig. 6·4

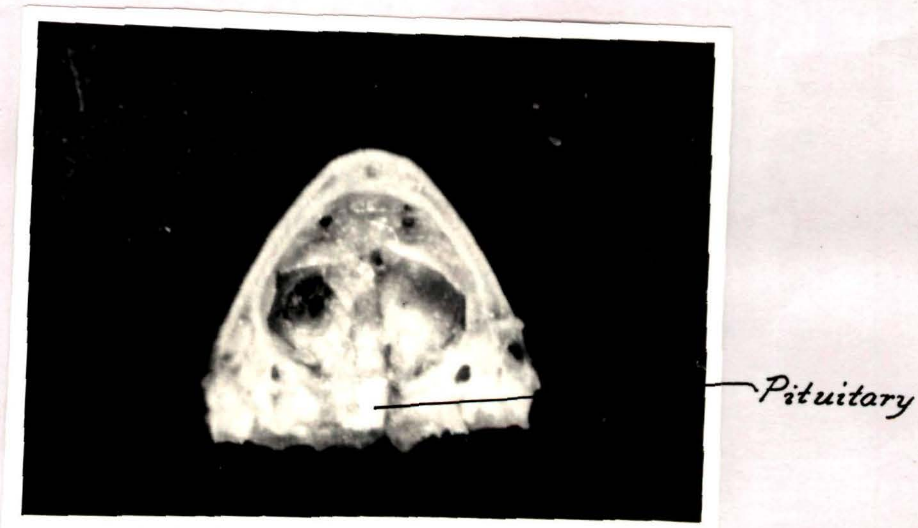


Fig. 6·5

Preparation of pituitaries homogenate and injection technique:

Mature healthy female (SVL 5.8 cm weight more than 25.0 gm) with enlarged abdomen were selected for experiment and placed separately in 2 litre clean and sterilized corning beakers, with some pond water. Required amount of pituitaries were homogenized in 0.5 ml distilled water in a sterilized crucible or glass homogenizer and the homogenate was injected with sterilized 2.0 ml hypodermic syrige fitted with number 21 needle in the dorsal femoral lymph sac of the frog (Fig. 6.6). The needle was withdrawn slowly using cotton swab dipped in 70% alcohol, with a finger tip and at the point of injection to prevent loss of injected homogenate from the lymph sac. The injected females were replaced in the beaker. (Fig. 6.7)

Stripping of ova and fertilization:

The stripping was attempted about 20 hours of the injection. The frog was held in left hand and gentle pressure and backward movement was applied with the palm of right hand. As soon as some eggs were seen coming out of the cloaca, the frog was again placed back in the beaker and sperm suspension were quickly prepared by macerating 4 testis of mature males in 200 ml of pond water in finger bowls. The sperm suspension was checked under microscope

Fig. 6·6

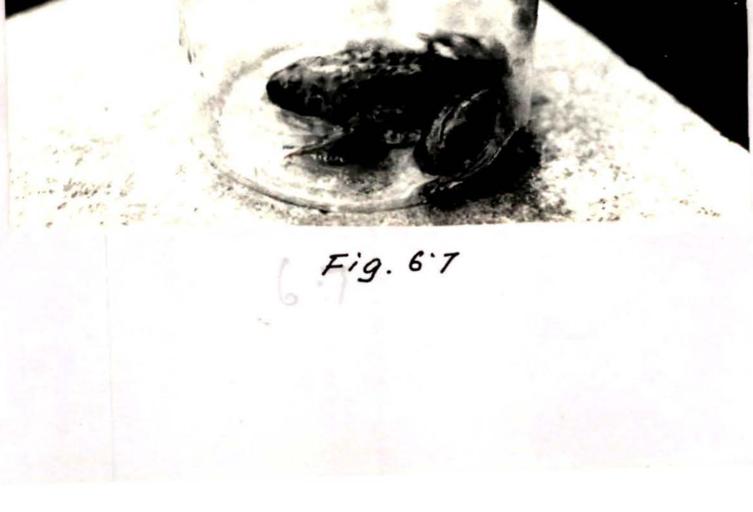


Fig. 6·7

and as soon as the sperms regain the mobility, eggs from the tested female were stripped into the sperm suspension. First cleavage was taken as the test for the successful fertilization.

Fecundity and gonado somatic index:

For fecundity estimations, both ovaries, of the frogs, collected and preserved during different months were taken out, washed and blotted on the filter papers to remove the excessive moisture. Extraneous, tissues were removed from each ovary under binocular microscope. The volume of the ovaries was measured with the help of measuring cylinder, through water displacement technique. Thereafter the ovaries were divided into four aliquots and numbers of ova in each were counted, to find out the total numbers of ova in the ovaries of each frog. Numbers of eggs in the ovaries of each frog and its total length/weight were plotted to prepare scatter graph and regression was worked out to find out the fecundity. Similarly, with the help of scatter diagram and regression equation the relationship between the SV length and weight of the ova has also been calculated. Terentjev's (1960) emperical formula for the calculation of fecundity has also been applied on its log 'n' and log 'e' bases.

The gonado-somatic index for each frog has been calculated as:

$$\text{Gonado-somatic index} = \frac{\text{Weight of ova}}{\text{Weight of frog}} \times 100$$

(7.2) EXPERIMENTS AND RESULTS

In 1977, some pilot experiments were performed to find out the approximate dosage (see Table 6.1), in which, 3 - 8 pituitaries were homogenated in 0.5 ml distilled water and were administered in the frogs weighing from 29.50 gm to 32.50 g (SV length 5.85 cm - 6.10 cm). It was observed that a doage of about 0.08 mg/g weight of the female caused successful induction of ovulation and spawning.

Subsequently it was confirmed that females having SV length 5.8 cm or above and weight more than 25.0 g responds to pituitary injection and others do not. A general observations was that less agile females with enlarged abdomen and while at rest keeping hind limbs extended widely, show better response to pituitary injection than others.

Based on the preliminary findings, following experiments were performed:

1) Experiments with fresh homoplastic pituitaries.
2) Experiments with homoplastic pituitaries preserved in absolute ethyl alcohol.
3) Experiments with homoplastic pituitaries preserved for one to two years.

Table 6.1

Pilot experiments to calculate the dosage of homoplastic pituitaries for induced ovulation in *Rana cyanophlyctis*

Exp. No.	Injection		Atm. Temp. °C	Pituitary			Pituitary weight/ weight of injected female mg/g	Volume of homogenate injected	Condition of female		Stripping		Number of ova obtained
	Date	Time		Sex	Number	Weight g			SVL cm	Weight g	Date	Time	
1_a	8.6.77	11.30	21.5	M	4	0.0126	0.05	0.5	5.45	23.40	-	-	-
1_b	8.6.77	12.00	21.5	F	3	0.0148	0.05	"	5.85	29.50	-	-	-
1_c	8.6.77	12.20	21.5	F	4	0.0204	0.06	"	6.10	32.10	-	-	-
1_d	8.6.77	12.45	21.5	F	5	0.0264	0.08	"	6.00	30.70	9.6.77	8.30	105
1_e	8.6.77	12.40	21.5	F	6	0.0313	0.10	"	5.90	31.20	9.6.77	6.00	193
1_f	8.6.77	16.40	21.5	F	8	0.0431	0.15	"	6.05	32.20	9.6.77	6.40	79

4) Experiments with heteroplastic pituitaries.

5) Experiments with homoplastic pituitaries collected from immediately dead frogs.

6) Experiments to see induction of ovulation second time.

Experiments with fresh homoplastic pituitaries:

The female frogs of SV length varying from 5.80 cm to 6.20 cm and weight 26.00 gm- 32.90 gm, were injected with the homogenate made up of 5 to 8 freshly collected homoplastic males and female pituitaries, of the weight varying from 0.0296 gm to 0.15 gm during June and July months, at laboratory temperature varying between 20.8°C to 21.5°C. The data has been compiled in Table 6.2

On the next day, after a lapse of 14-24 hours stripping were achieved in all the experiments frogs. The number of ova released varied from 29 to 196 in different experiments. It was noted that male and female freshly collected homoplastic pituitaries are capable of inducing ovulation in frogs and a dosage of 0.1 mg/gm body weight leads to successful spawning during rainy season.

Experiments with homoplastic pituitaries freshly preserved in absolute ethyl alcohol.

The female frogs having SV length 4.9 cm to 6.2 cm and weight between 11.70 gm and 32.00 were injected with

Table 6.2

Experimental breeding with fresh pituitaries in Rana cyanophlyctis

Exp. No.	Injection Date	Injection Time	Atm. Temp. °C	Pituitary Sex	Pituitary Number	Pituitary Weight gm	Pituitary weight/ weight of female frog mg/gm	Volume of homogenate injected ml	Condition of female SVL cm	Condition of female Weight gm	Stripping Date	Stripping Time	Number of ova obtained
2_a	21.6.77	19.10	21.5	F	5	0.0384	0.1	0.5	6.10	31.00	22.6.77	12.10	78
*2_b	21.6.77	20.00	21.5	F	6	0.0391	0.1	0.5	6.20	32.70	22.6.77	9.00	29
*2_c	6.7.78	11.00	20.8	F	6	0.0343	0.1	0.5	6.10	32.90	7.7.78	8.10	176
*2_d	6.7.78	11.30	20.8	F	5	0.0386	0.15	0.5	5.80	26.00	7.7.78	10.20	71
*2_e	15.7.78	14.10	21.2	F	5	0.0361	0.1	0.5	6.20	32.60	16.7.78	10.40	196
2_f	15.7.78	14.25	21.2	M	8	0.0298	0.1	0.5	5.85	30.00	16.7.78	13.00	67

* Frogs subjected to homoplastic injection for second time.

the homogenate made up of 3 to 8 male and female preserved homoplastic pituitaries of the weight varying from 0.0138 to 0.0397 gm, during July at an approximately 21.0°C to 22.0°C laboratory temperature. The dosage of pituitary homogenate of approximately 0.1 mg/gm body weight were successful to induce ovulate the mature frog. The data has been tabulated in Table 6.6. Out of the six experiments performed Exp. No.6_a , 6_b and 6_f were successful, whereas experiments No. 6_c, 6_d and 6_e were unsuccessful. These results indicate that Rana cyanophlyctis of size group 5.9 cm and above and weight 30.0 gm and above, could be induced breed upon freshly preserved homoplastic pituitaries.

Experiments with homoplastic pituitaries preserved for one year in absolute ethyl alcohol:

These series of experiments were performed during June and July (Laboratory temperature 21.5 - 22.5°C) with homoplastic pituitaries preserved for one year in absolute ethyl alcohol. It was observed that the pituitaries of Rana cyanophlyctis remain potent in absolute ethyl alcohol for one year. The pituitary dosage of 0.1 mg/gm weight of the female were found to be effective. The data has been compiled in Table 6.7. It is further noted that the skipper frog having SV length 5.8 cm in length and

Table 6.6

Experiments performed with the freshly preserved homoplastic pituitaries in *Rana cyanophlyctis*

Exp. No.	Injection		Atm. Temp. °C	Pituitary			Pituitary weight/ weight of female frog mg/gm	Volume of homogenate injected ml	Condition of female		Stripping		Number of ova obtained
	Date	Time		Sex	Number	Weight gm			SVL cm	Weight gm	Date	Time	
6_a	21.7.77	16.00	22.0	F	5	0.0296	0.09	0.5	5.90	30.00	22.7.77	12.30	79
6_b	21.7.77	16.30	22.0	F	6	0.0309	0.1	0.5	6.10	32.00	22.7.77	11.00	28
6_c	21.7.77	16.45	22.0	M	5	0.0138	0.1	0.5	4.90	11.70	22.7.77	-	-
6_d	21.7.77	17.10	22.0	F	3	0.0148	0.1	0.5	5.20	15.50	22.7.77	-	-
6_e	21.7.77	17.35	22.0	M	8	0.0338	0.15	0.5	5.60	20.50	22.7.77	-	-
6_f	21.7.77	18.00	22.0	M	8	0.0397	0.1	0.5	6.20	31.90	22.7.77	12.10	112

Table 6.7

Experiments of induced breeding with homoplastic pituitaries preserved for one year in *Rana cyanophlyctis*

(Pituitaries preserved on 19.6.77)

Exp. No.	Injection Date	Injection Time	Atm. Temp. °C	Pituitary Sex	Pituitary Number	Pituitary Weight gm	Pituitary weight/weight of female frog mg/gm	Volume of homogenate injected ml	Condition of female SVL cm	Condition of female Weight gm	Stripping Date	Stripping Time	Number of ova obtained
7_a	16.6.78	17.00	21.5	F	7	0.0313	0.1	0.5	6.00	29.20	17.6.78	15.10	66
7_b	16.6.78	17.20	21.5	F	6	0.0288	0.1	0.5	5.80	28.00	17.6.78	15.30	47
7_c	10.7.78	18.00	22.5	M&F	6	0.0294	0.1	0.5	6.25	29.10	11.7.78	18.10	209
7_d	10.7.78	18.15	22.5	M	8	0.0312	0.15	0.5	5.55	22.20	-	-	-
7_e	10.7.78	18.40	22.5	M	8	0.0301	0.15	0.5	5.10	22.40	-	-	-

29.20 gm in weight have only responded to one year preserved pituitaries, the experiments 7_d and 7_e (see Table 6.7) were found unsuccessful, due to immature size and under weight of the experimental frogs.

Experiments with homoplastic pituitaries preserved for two years in absolute ethyl alcohol:

The present series of experiments were performed in the months of May and June 1979 (laboratory temperature 20.6-22.0°C) with the pituitaries of Rana cyanophlyctis preserved in absolute ethyl alcohol since July 1977 (two years). During preservation the portion of absolute ethyl alcohol evaporated was replaced by fresh one. The required dosage of such pituitaries were homogenised in 0.5 ml glass distilled water and injected in the dorsal lymp sac of the female frogs. The data has been compiled in Table 6.8. It was observed that the pituitaries preserved in absolute ethyl alcohol retained its potency for a minimum of 2 years and act as efficiently as fresh or one year old preserved pituitaries. In this series, all the six experiments were found successful. The dosages during the experiments was observed varying between 0.08 mg/gm to 0.09 mg/gm weight of the female.

Table 6.8

Experimental breeding with homoplastic pituitaries preserved for two years in <u>Rana cyanophlyctis</u>

(Pituitaries preserved on 8.5.77)

Exp. No.	Injection Date	Injection Time	Atm. Temp. °C	Pituitary Sex	Pituitary Number	Pituitary Weight gm	Pituitary weight/ weight of female frog mg/gm	Volume of homogenate injected ml	Condition of female SVL cm	Condition of female Weight gm	Stripping Date	Stripping Time	Number of ova obtained
8_a	19.5.79	17.30	22.0	M	8	0.0316	0.09	0.5	6.30	34.40	20.5.79	9.00	167
8_b	19.5.79	17.50	22.0	M	9	0.0343	0.1	0.5	6.20	33.35	20.5.79	11.30	269
8_c	19.5.79	18.10	22.0	F	6	0.0309	0.09	0.5	5.95	32.00	20.5.79	12.20	121
8_d	19.5.79	18.25	22.0	F	6	0.0314	0.1	0.5	6.15	32.16	20.5.79	9.40	197
8_e	9.6.79	18.00	20.6	M&F	7	0.032	0.09	0.5	6.25	34.60	5.6.79	10.30	345
8_f	4.6.79	19.10	20.6	M&F	6	0.0268	0.09	0.5	5.90	29.80	5.6.79	9.10	205

Experiments with the help of preserved homoplastic pituitaries in different months:

Induced breeing was attempted in Rana cyanophlyctis for 14 continuous months, from June 1977 to July 1978. In all the 43 experiments performed, the preserved homoplastic pituitaries were used. Data has been compiled in Table 6.9. The observation made are as follows:

1. Induction of ovulation was achieved in the frogs having a measurement SV length 5.8 cm or over and weight 25.0 gm and over. Female frogs smaller in size and weight did not respond to pituitary injection.

2. A dosage of 0.08 - 0.2 mg/gm weight of the female was found effective to achieve ovulation in Rana cyanophlyctis. A slightly increased dosage of 0.15 mg/gm - 0.2 mg/gm was found to be effective during March/April and September/October months (Pre and Post breeding phase) and approximately 0.08 mg/gm was found effective during peak rainy season May/June (breeding phase) this also coincides with its peak active phase (see Chapter 3).

3. Although the frogs could be induced ovulated in each month from March till October, but the amounts of egg laid ovulated were found varying in different months. The maximum numbers of 667 ova were achieved in the frog from May month and minimum of 27 ova from the frog in March month. The frogs collected and

Table 6.9

Experimental breeding in *Rana cyanophlyctis* with the help of homoplastic pituitaries during every month from June 1977 till August 1978

Exp. No.	Injection Date	Injection Time	Atm. Temp. °C	Pituitary Sex	Pituitary Number	Pituitary Weight gm	Pituitary weight/ weight of female frog mg/gm	Volume of homogenate injected ml	Condition of female SVL cm	Condition of female Weight gm	Stripping Date	Stripping Time	Number of ova obtained
1.	14.6.77	16.10	21.5	F	6	0.0391	0.2	0.5	5.40	17.70	-	-	-
2.	21.7.77	13.10	22.8	F	5	0.0296	0.1	"	5.90	30.00	22.7.77	15.30	79
3.	21.7.77	13.30	22.8	M	7	0.0309	0.1	"	6.10	32.00	22.7.77	12.45	28
4.	21.7.77	13.55	22.8	M	4	0.0138	0.1	"	4.90	11.70	-	-	-
5.	21.7.77	14.20	23.4	M	5	0.0148	0.1	"	5.20	15.50	-	-	-
6.	21.7.77	14.45	23.4	F	6	0.0338	0.15	"	5.60	20.50	-	-	-
7.	14.8.77	12.30	23.4	F	7	0.0425	0.2	"	5.40	19.70	-	-	-
8.	14.8.77	13.10	23.4	F	7	0.0412	0.2	"	5.55	21.70	-	-	-
9.	14.8.77	14.00	23.4	M	8	0.0312	0.15	"	5.35	17.50	-	-	-
10.	14.8.77	14.30	23.4	M	8	0.0432	0.15	"	5.85	27.50	15.8.77	17.10	29
11.	8.9.77	16.20	22.0	F	5	0.0310	0.15	"	5.45	20.60	-	-	-
12.	8.9.77	16.40	22.0	M	6	0.0260	0.1	"	5.75	26.00	-	-	-
13.	8.9.77	17.10	22.0	F	7	0.0430	0.15	"	5.80	26.00	9.9.77	13.30	65
14.	8.9.77	17.35	22.0	F	5	0.0382	0.1	"	6.05	29.50	9.9.77	12.45	32
15.	4.10.77	10.40	21.5	F	7	0.0406	0.15	"	5.85	27.80	5.10.77	12.10	29
16.	4.10.77	11.10	21.5	M	8	0.0316	0.2	"	5.25	15.00	-	-	-
17.	4.10.77	11.35	21.5	M	8	0.0364	0.15	"	5.80	26.50	-	-	-
18.	4.10.77	12.10	21.5	M	8	0.0386	0.1	"	6.00	29.70	-	-	-
19.	11.11.77	10.40	18.0	F	5	0.0316	0.1	"	5.90	28.40	-	-	-
20.	11.11.77	11.20	18.2	F	5	0.0338	0.15	"	5.50	21.60	-	-	-
21.	18.12.77	12.10	17.8	F	6	0.0356	0.1	"	6.30	29.90	-	-	-
22.	18.12.77	17.30	17.8	M	7	0.0297	0.1	"	5.75	26.35	-	-	-
23.	7.1.78	11.30	15.2	F	6	0.0342	0.1	"	5.80	28.60	-	-	-
24.	7.1.78	11.55	15.2	M	7	0.0237	0.1	"	5.45	20.65	-	-	-
25.	9.2.78	10.40	16.8	F	5	0.0284	0.1	"	5.60	24.60	-	-	-
26.	9.2.78	11.20	16.8	F	6	0.0311	0.15	"	5.30	20.35	-	-	-
27.	9.2.78	11.40	16.8	F	5	0.0278	0.1	"	5.55	24.70	-	-	-
28.	19.3.78	10.40	18.0	M	6	0.0272	0.09	"	5.95	30.20	20.3.78	16.10	34
29.	19.3.78	11.10	18.0	F	6	0.0370	0.1	"	5.90	28.50	20.3.78	16.40	27
30.	19.3.78	11.30	18.0	F	6	0.0341	0.15	"	5.30	20.40	-	-	-
31.	14.4.78	10.20	18.7	F	6	0.0314	0.1	"	5.90	27.40	15.4.78	12.30	82
32.	14.4.78	10.50	18.7	F	8	0.0544	0.2	"	6.05	29.10	15.4.78	10.45	32
33.	3.5.78	12.10	18.8	F	6	0.0322	0.1	"	5.85	26.10	4.5.78	14.15	471
34.	3.5.78	12.40	18.8	F	8	0.0601	0.2	"	6.05	29.00	-	-	-
35.	12.5.78	12.15	20.4	M	7	0.0338	0.1	"	6.15	33.50	-	-	-
36.	12.5.78	12.30	20.4	F	7	0.0398	0.1	"	6.40	34.50	12.5.78	10.20	271
37.	12.5.78	13.00	20.4	M	7	0.0261	0.08	"	6.20	29.50	12.5.78	11.10	181
38.	19.5.78	13.40	19.6	M	6	0.0261	0.08	"	5.90	28.60	20.5.78	9.10	667
39.	19.5.78	14.10	19.6	M	6	0.0251	0.09	"	5.90	26.90	20.5.78	9.30	133
40.	6.6.78	15.30	21.7	F	6	0.0358	0.1	"	6.10	32.70	7.6.78	9.45	170
41.	6.6.78	16.05	21.7	F	6	0.0312	0.1	"	5.95	27.40	7.6.78	10.05	120
42.	16.6.78	15.10	21.9	F	6	0.0313	0.1	"	6.00	29.20	17.6.78	10.40	66
43.	16.6.78	15.40	21.9	M	7	0.0289	0.1	"	5.80	25.00	17.6.78	11.55	47
44.	10.7.78	14.30	20.8	M	7	0.0294	0.1	"	6.20	23.00	11.7.78	14.15	209
45.	10.7.78	15.05	20.8	M	8	0.0312	0.15	"	5.55	22.20	-	-	-
46.	10.7.78	15.30	20.8	F	5	0.0301	0.15	"	5.60	26.40	-	-	-
47.	14.7.78	10.30	22.1	M	7	0.0262	0.1	"	6.30	29.80	15.7.78	12.30	31
48.	14.7.78	11.05	22.1	F	6	0.0316	0.1	"	6.10	30.10	-	-	-

induced breed during May and June months were the best in obtaining number of ova and the percentage of success in experimental breeding. Further, the maximum success in the ovulation were observed in the month, when it rained heavily and when the abundance and the activities of the frog on the land were maximum.

4. No ovulation could be achieved during winter months from late October till late February, perhaps because of physiological inertness in adult frogs.

5. The frogs (serial number 34, 35, 45, 47, 48 of Table 6.9) were collected and stored in plastic jar for a week, and then were subjected to induced breeding. Out of 5 frogs only one (serial No. 47) could ovulate with a poor spawn of 31. Hence, it can be concluded that the frogs stored for over one week without proper feeding in the laboratory cannot be induced ovulated.

Experiments with heteroplastic pituitaries of *Rana limnocharis*:

This series of experiments, were performed with freshly collected and absolute ethyl alcohol preserved

Table 9.4

Experimental breeding in *Rana cyanophlyctis* with the help of heteroplastic pituitaries of *Rana limnocharis*

Exp. No.	Injection Date	Injection Time	Atm. Temp.	Pituitary Sex	Pituitary Number	Pituitary Weight g	Pituitary weight/ weight of injected female mg/g	Volume of homogenate injected	Condition of female SVL cm	Condition of female Weight g	Stripping Date	Stripping Time	Number of ova obtained
4_a	28.5.77	9.00	21.4	F	9	0.0219	0.1	0.5	5.30	20.8	-	-	-
4_b	28.5.77	9.30	21.4	F	12	0.0279	0.1	"	5.80	26.6	29.5.77	12.20	98
4c	28.5.77	9.55	21.4	F	7	0.0186	0.08	"	5.40	22.4	-	-	-
4_d	2.6.78	9.30	20.8	M	14	0.0305	0.09	"	6.25	32.0	3.6.77	11.45	100
4_e	2.6.78	9.30	20.8	M	15	0.0335	0.08	"	6.40	40.0	3.6.77	12.30	109
4_f	2.6.78	9.30	20.8	M	13	0.0295	0.08	"	6.35	35.0	3.6.77	14.30	88
4_g	2.6.78	9.30	20.8	M+F	13	0.0431	0.1	"	6.60	40.0	3.6.77	16.20	36
4_h	2.6.78	9.30	28.7	M+F	9	0.0388	0.1	"	6.30	38.0	3.6.77	14.05	43
4_i	14.6.77	20.5	20.6	F	19	0.0865	0.2	"	6.45	41.0	-	-	-
4_j	14.6.77	20.5	20.6	F	13	0.0783	0.3	"	5.30	21.5	-	-	-

pituitaries of Rana limnocharis. Data has been compiled in Table 6.4. It was observed that a dosage of 0.1 mg/gm to 0.15 mg/gm weight of the females of Rana cyanophlyctis were found effective to achieve ovulation in the frogs. In the experimental frogs 6.4_i and 6.4_j did not respond to ovulation and died. The failure is attributed to higher dosage of pituitary injection. However, in the above series of experiments a minimum of 36 ova and a maximum of 109 ova (see Exp. No. 6.4_g and 6.4_e of Table 6.4) were procured.

Experiments of induce ovulations with the homoplastic pituitaries collected from dead Rana cyanophlyctis :

In the present series of experiments the pituitaries collected from the frogs (male and female) died for 12 hours to 18 hours were homogenated and injected with 0.5 ml of distilled water. The data has been compiled and presented in Table 6.3. 4 out of such 6 experiments performed were found successful. It was observed that a pituitary dosage of 0.09 mg/gm female body weight leads to successful ovulation. A minimum of 67 ova and maximum of 170 ova can thus be obtained. Further, it can be concluded that the frog although dies, its pituitary retains the gonadotrophic activities for some time over 18-20 hours.

Table 6.3

Experiments of induced breeding in <u>Rana cyanophlyctis</u> with the help of homoplastic pituitaries collected from the frog died 4 to 6 hr before of experimentation

Exp. No.	Injection Date	Injection Time	Atm. Temp. °C	Pituitary Sex	Pituitary Number	Pituitary Weight g	Pituitary weight/ weight of injected female mg/g	Volume of homogenate injected	Condition of female SVL cm	Condition of female Weight g	Stripping Date	Stripping Time	Number of ova obtained
3_a	24.5.78	18.40	20.4	M	6	0.0235	0.09	0.5	5.80	30.50	-	-	-
3_b	24.5.78	19.10	20.4	M	7	0.0299	0.1	"	5.35	22.80	-	-	-
3_c	6.6.78	15.30	19.8	F	6	0.0298	0.09	"	6.25	33.70	7.6.78	9.30	170
3_d	6.6.78	15.45	19.8	F	4	0.0198	0.07	"	6.15	29.40	7.6.78	11.30	120
3_e	10.6.78	13.10	21.6	F	5	0.0203	0.08	"	5.85	29.20	11.6.78	9.00	66
3_f	10.6.78	14.00	21.6	F	7	0.0199	0.07	"	5.70	26.00	11.6.67	11.30	147

Experiments of induce ovulations in the frogs which has already ovulated once:

The frogs which has already ovulated with the help of homoplastic fresh pituitaries, once (Table 6.2) have been subjected to the experimental breeding for the second time, with the help of preserved homoplastic pituitaries. The data of the experiments have been compiled and presented in Table 6.5. Only the asterisk marked experimental frogs (Table 6.2) which have once ovulated with the help of fresh homoplastic pituitaries extract were found to be reovulating with preserved homoplastic pituitary extract, and it is noted that the frog No.6.2_b which ovulated 29 ova on 22.6.1977 re-ovulated 100 ova on 25.6.1977 (see Exp. frog No. 6.5_b). The experimental frog No. 2 (Table 6.2) which ovulated 176 ova on 7.7.1978, on induction re-ovulated 68 ova on 9.7.1978 (see Exp. frog No. 5_c); the experimental frog No. 2_d (Table 6.2) which ovulated 71 ova on 7.7.1978 induction re-ovulated 49 ova on 9.7.1978 (see Exp. frog No. 6.5_d) and finally experimental frog No. 2 (Table 6.2) which ovulated 196 ova on 16.7.1978 on induction re-ovulated 72 ova on 18.7.1978 (see Exp. frog No. 6.5_e). Hence, it can be concluded that the frogs, that has already induced bred once can be induced to breed for the second time, with a short gap for second injection.

Table 6.5

Experiments of induced breeding in <u>Rana cyanophlyctis</u> for the second time, that has already induced once

Exp. No.	Injection		Atm. Temp. °C	Pituitary			Pituitary weight/ weight of injected female mg/g	Volume of homogenate injected	Condition of female		Stripping		Number of ova obtained
	Date	Time		Sex	Number	Weight g			SVL cm	Weight g	Date	Time	
5_a	23.6.77	18.10	20	F	5	0.0329	0.1	0.5	6.20	32.70	24.6.77	18.40	100
5_b	23.6.77	17.20	20	F	6	0.0337	0.1	"	6.20	31.00	-	-	-
5_c	18.7.78	13.10	20.6	F	5	0.0313	0.1	"	6.10	32.90	19.7.78	8.10	68
5_d	18.7.78	13.40	20.6	M	7	0.0261	0.1	"	5.80	25.50	19.7.78	12.30	49
5_e	17.7.78	14.30	19.2	M	9	0.0298	0.09	"	6.20	32.60	18.7.78	19.50	72
5_f	17.7.78	20.40	19.2	M	9	0.0287	0.09	"	5.85	29.85	18.7.78	-	-

Gonado sometic index:

The Gonado somatic index, the ratio between the weight of the gonad and the weight of the animal has been calculated in 20 female frogs of SV length and body weight ranging from 5.45 cm to 7.15 cm and 18.17 gm to 49.02 gm respectively (Table 6.10).

The graph plotted between SV length versus weight of ova (Table 6.10; Fig. 6.2) showed a linear relationship and is expressed as:

$$Y = mX + c$$

where

Y & X - represent variables,
X & Y represent weight of ova and SV length respectively.

m & c - constants of the equation.

The relationship between weight of ova and SV length of frog has been calculated, applying the sum of least square method and was noted as follows:

$$\log W_1 = -4.506 + 6.4369 \log L$$

(See Fig. 6.2)

where

W_1 = weight of ova

L_1 = SV length of frog

Fig. 6.1 (A) : Relationship between Body weight of female *Rana cyanophlyctis* and gonadosomatic index.

6.2 (B) : Relationship between ova weight of female *Rana cyanophlyctis* and gonado somatic index

6.1 (C) : Relationship between SV length of female and gonadosomatic index.

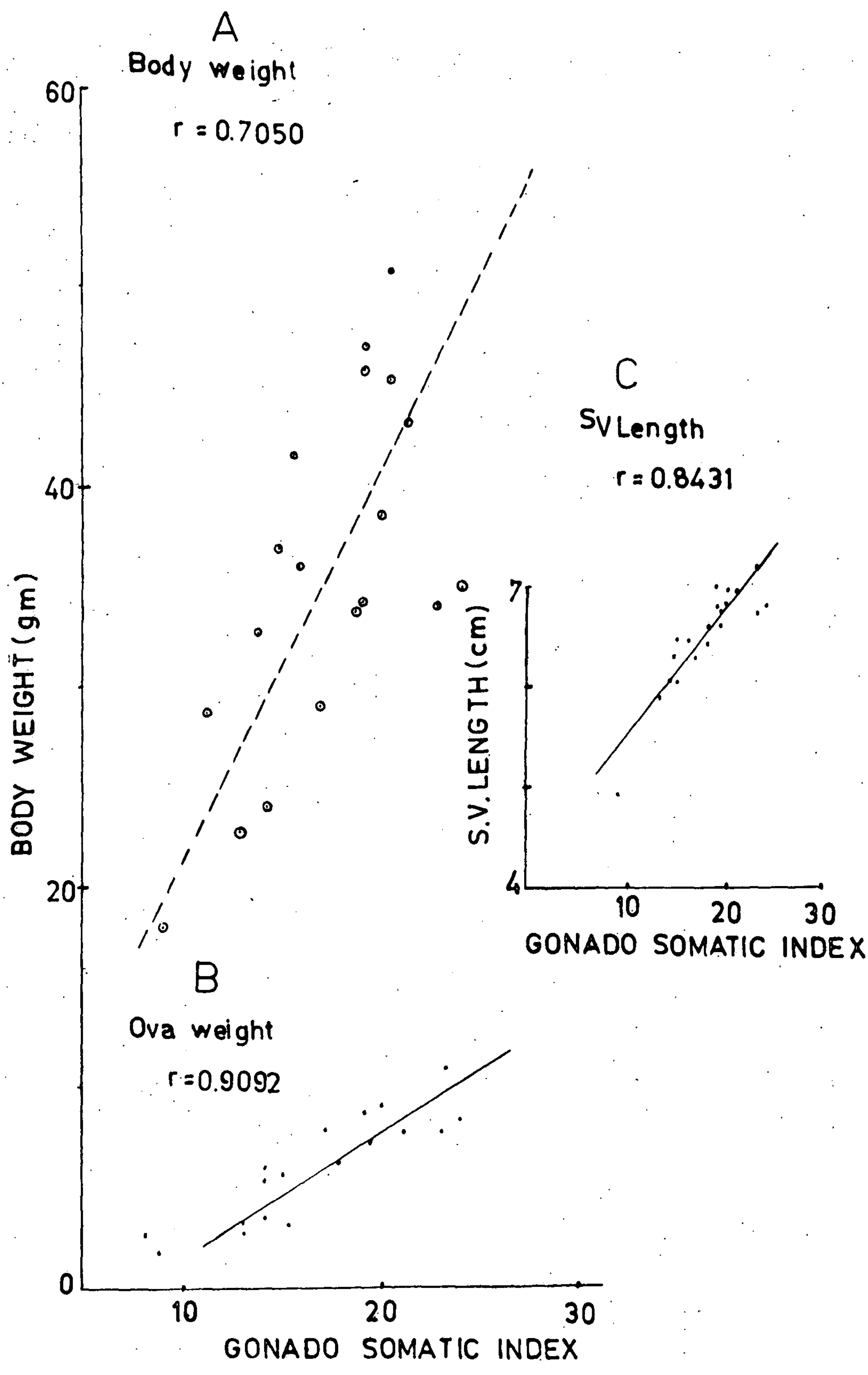

A
Body weight
r = 0.7050
BODY WEIGHT (gm)
60
40
20
0
B
Ova weight
r=0.9092
10
20
30
GONADO SOMATIC INDEX
C
SV Length
r = 0.8431
S.V. LENGTH (cm)
7
4
10
20
30
GONADO SOMATIC INDEX

Fig. 6.1

Table 6.10

Relationship of SV length and weight of frog with number, weight of ova and gonado somatic index in <u>Rana cyanophlyctis</u>

S. No.	Body's Length cm	Body's Weight gm	OVA Weight Measured gm	OVA Weight Calculated gm	OVA Number By counts	OVA Number By regression equation derived	OVA Number Tarentjev's equation at $\log_e$	OVA Number Tarentjev's equation at $\log_n$	Gonado somatic index
1.	5.45	18.17	1.6291	1.7119	1262	1159	6900	713	8.9649
2.	5.70	22.96	3.0121	2.2856	1846	1522	7604	786	13.1205
3.	6.10	24.35	3.4355	3.5343	1955	2300	8088	910	14.1088
4.	6.10	28.10	2.2871	3.5343	1531	2300	8088	910	8.1390
5.	6.50	36.82	5.2712	5.3198	4118	3386	10108	1042	14.3138
6.	6.55	37.45	5.7211	5.5873	4236	3548	10277	1061	15.4228
7.	6.60	33.92	6.4286	5.8667	3866	3716	10448	1079	18.9522
8.	6.60	33.96	6.2982	7.1138	3921	3716	10448	1079	18.5437
9.	6.75	36.94	6.2516	6.7842	3985	4260	10931	1133	16.9236
10.	6.75	34.16	7.8954	6.7842	5197	4260	10931	1133	23.1072
11.	6.80	37.45	7.1418	7.1138	4187	4456	11147	1157	19.0702
12.	6.80	37.17	6.7456	7.1138	4231	4456	11147	1157	18.1483
13.	6.85	46.08	8.9653	7.4593	4745	4659	11325	1167	19.4540
14.	6.95	35.43	8.5835	8.1884	5580	5089	11686	1207	24.2263
15.	6.95	38.36	8.0763	8.1884	5275	5089	11686	1207	21.0530
16.	7.00	38.96	8.1263	8.5743	5561	5316	11869	1226	20.8542
17.	7.05	45.63	9.1762	8.9764	4982	5551	12054	1245	20.1081
18.	7.05	47.27	8.9985	8.9764	6695	5551	12054	1245	19.0345
19.	7.15	43.53	8.1276	9.8265	4465	6048	12427	1283	18.6699
20.	7.15	49.02	11.5879	9.8265	5808	6048	12427	1283	23.6374

The weight of each frog has been calculated in relation to its SV length with the help of above derived relationship. The frog of SV length measuring 7.15 cm showed maximum weight of ovary 9.8265 gm and the frog, with SV length 5.45 cm showed minimum weight of ovary 1.7119 gm (Table 6.10). However, the weight of ova measured through aliquots showed, the minimum ovary weight (1.6291 gm) female frog of SV length 5.45 cm and maximum ovary weight(11.5879 gm) in female frog of SV length 7.15 cm. The correlation coefficient drived between SV length and weight of ovary was found 0.9581 (Fig. 6.2), highly significant at 1% and 5% levels.

Table 6.10 also show the comparison between the measured and calculated weight of ova (see Fig. 6.3) and measured and calculated members of ova (see Fig.6.3). The comparison reveals little variation in observed and calculated values. The scatter diagram plotted between gonado somatic index, and the body weight of the frogs; gonado somatic index and ovary weight of the frog and gonado somatic index and snout vent length of the frog have been best expressed by linear regression (Fig. 6.1 A,B,C). The correlation coefficient between gonado somatic index and body weight; gonado somatic index and ovary weight and gonado somatic index and snout-vent length of female frogs have also been calculated and were found to be 0.7050; 0.9092 and 0.8431, respectively.

Fecundity:

The graph plotted between log of SV length and log, numbers of ova, female frogs (Table 6.10) showed a linear relationship and can be expressed by the formula:

$$Y = mX + c$$

where

Y & X = represent variables namely ova number and SVL respectively.

m & c = equation constants

The relationship between fecundity and SV length of the frog has been found out applying the sum of least square method, from the above linear expression:

$$\log F = -1.4169 + 6.0851 \log L$$

where

F = number of eggs in thousand

L = SV length in cm

The fecundity in each frog has been calculated with the help of the above equation. A maximum number of

Fig. 6.3 : Correlation coefficient and relationship between weight of frog and number of ova.

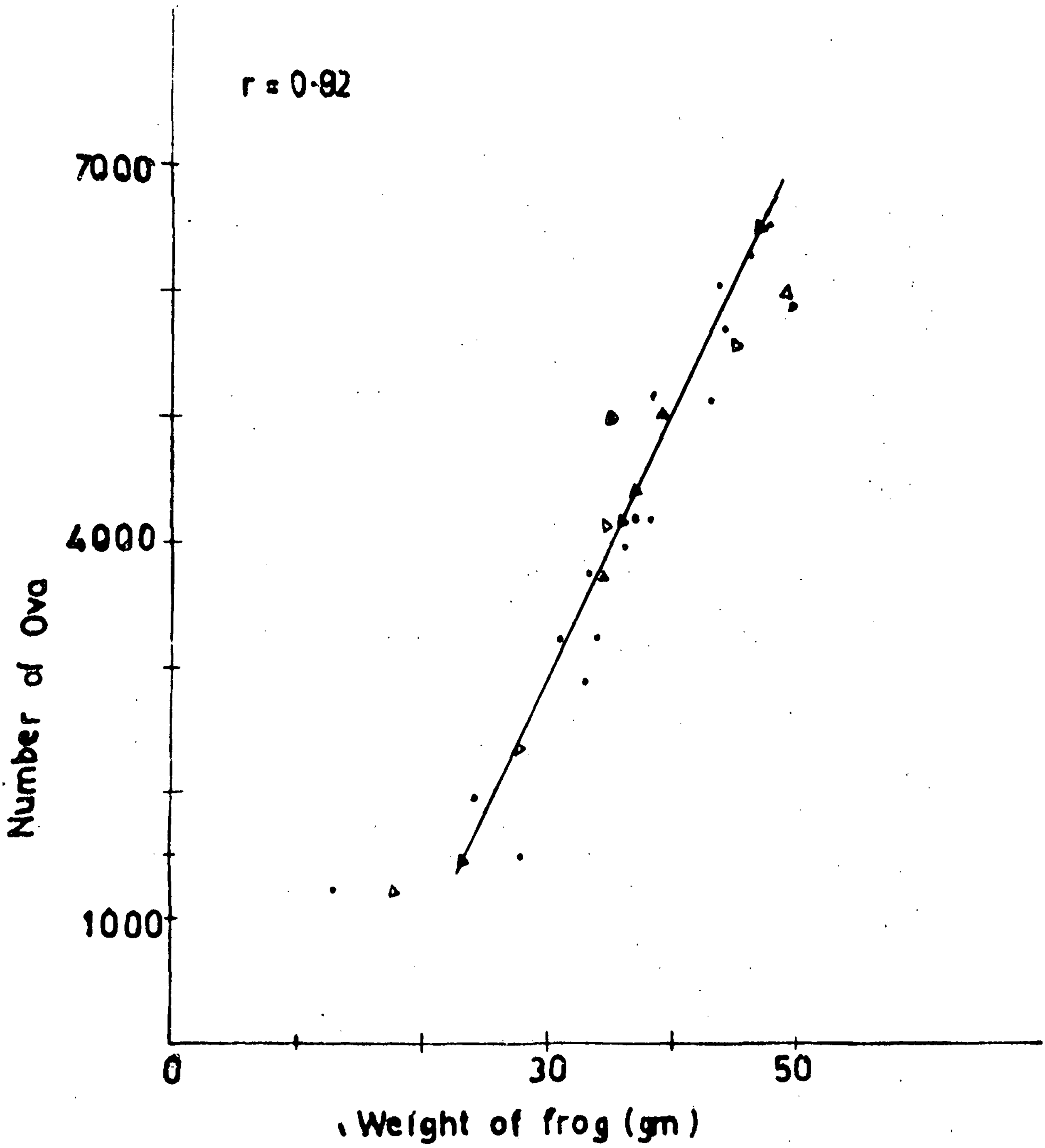


Fig. 6·3

6048 eggs were calculated from the frog of SV length 7.15 cm and minimum of 1159 eggs from the frog of SV length 5.45 cm (Table 6.10). However, the fecundity noted through gravimetric method showed the minimum count of 1262 eggs from the frog of SV length and weight of 5.45 cm and 18.17 gm respectively and maximum 6695 count at 7.05 cm and 47.27 gm respectively (Table 6.10). The correlation coefficient between the two variates length and fecundity was found to be 0.9432 (Fig. 6.2A) and was significant at 1% and 5% level of confidence.

The graph plotted between weight of ova and numbers of egg as 'Y' and 'X' axis showed linear expression [illegible]) with a significantly high correlation coefficient of 0.8628. The correlation coefficient derived between the weight of ova calculated [illegible] and number of ova (r = 0.9180) and was also found to be significant at 1% and 5% probability level.

Terentjev's (1960) emperical equation for the calculation of fecundity and clutch size for temperate frogs, has also been applied at $\log_e$ and $\log_n$ bases in Rana cyanophlyctis. The fecundity observed by gravimetric measurements, calculation by derived linear regression formula and by Terentjev's equations at $\log_e$ and $\log_n$ bases have been tabulated and compared in the Table 6.10,

Fig. 6.2 (A) : Linear relationship and regression equations between SV length and number of ova of *Rana cyanophlyctis*.

6.2 (B) : Regression equation, and linear relationship between SV length and weight of ova of *Rana cyanophlytis*.

6.2 (C) : Regression equation drawn at $\log_e$ in accordance to Terentjev's formula at given SVL of *Rana cyanophlyctis*.

6.2 (D) : Regression equation drawn at $\log_n$ in accordance to Terentjev's formula at given SVL of *Rana cyanophlyctis*.

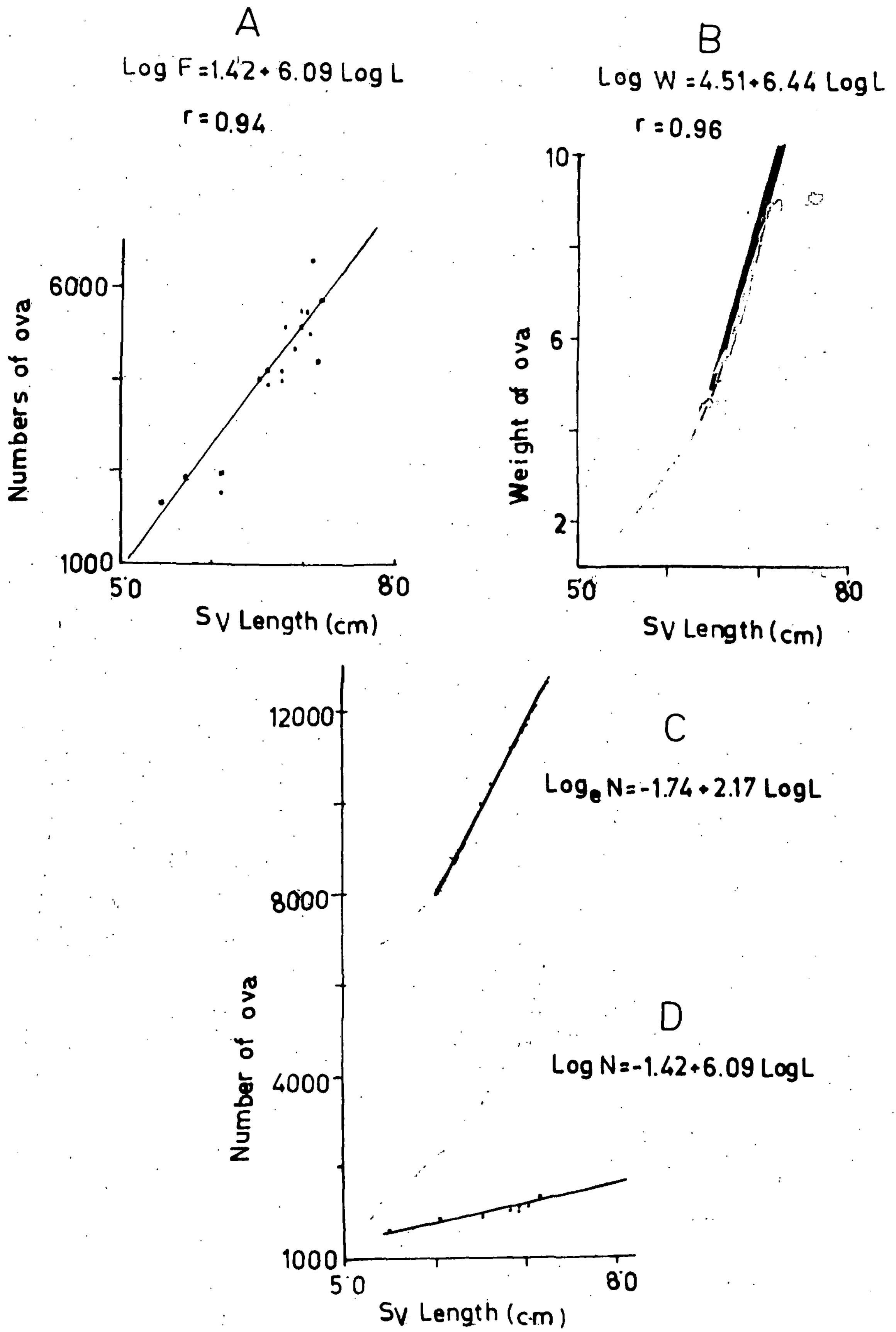

A
Log F =1.42 + 6.09 Log L
r = 0.94
Numbers of ova
6000
1000
50
80
SV Length (cm)
B
Log W =4.51+6.44 Log L
r = 0.96
10
6
2
Weight of ova
50
80
SV Length (cm)
12000
8000
4000
1000
Number of ova
C
Log_e N=-1.74+2.17 LogL
D
Log N=-1.42+6.09 LogL
50
80
SV Length (cm)

Fig. 6 2

and Fig.7.2 C and D. It is noted that fecundity calculated through Terentjev's equation does not hold good for Rana cyanophlyctis and varies greatly with the fecundity calculated by linear regression and gravimetric method.

DISCUSSION

Induced breeding by pituitary injection is now a routine procedure in many laboratories all over the world. Eversince, Ascheium and Zordek demonstration that pituitary implant enhances sexual development in mice (Allen, 1939) and Wolf's (1929) experiment that pituitary glands cause induction of ovulation in Rana pipiens, different techniques have been evolved for induced breeding (see Rugh, 1962; Nieuwkoop and Faber, 1967; Jhingran,1975). Nieuwkoop and Faber (1967) and Roy (1979) respectively described detailed technique of induce ovulation in Xenopus laevis and Rana limnocharis, following Rugh (1962) technique. The experiments in the present investigation on the induced breeding of Rana cyanophlyctis has been followed in accordance to the technique prescribed by Nieuwkoop and Faber (1967) and Roy (1979). Though Rugh

(1962) prescribed use of fresh pituitary, he did mention that pituitaries preserved in ethanol can be used for this purpose. However, Rugh's (1962) technique was found to be somewhat cumbersome as in every set of experiment one has to collect a number of frogs, decapitate some of them to collect required numbers of pituitaries, and then inject it along with some distilled water in the abdomen of the experimental female.

In fishes acetone preserved pituitaries are used (Alikunhi et al., 1960; Choudhuri, 1960, 1963 and Ibrahim and Choudhuri, 1966), but in frogs pituitaries preserved in absolute ethyl alcohol yield successful results. It is difficult to explain such differential response, in fishes and frogs. In the present investigation response to fresh, preserved as well as pituitaries taken out from just dead or dying frogs was tested. As reported earlier (Roy 1979; Khare, Roy and Kumar, 1981) the use of frog pituitaries preserved in absolute ethyl alcohol was found very convenient as they can be effectively used even after 2-3 years of preservation. This investigation reveal that :

(1) The frog can be induced breed for eight continuous months, namely March, April, May, June, July, August, September and October.

(2) The frog of minimum measurements of SV length 5.8 cm and weight 25.0 gm is required for successful induced spawning.

(3) The pituitary dosage of approximately 0.08 mg/g body weight for fully mature and slightly for somewhat immature female frogs can be effectively used for induced ovulation.

(4) The number of ova obtained by induced breeding during rainy season are more, maximum being 667, than at the beginning or end of rainy season, minimum being 24.

(5) At cold weather of Shillong induction of ovulation occurs in 20 to 24 hours.

(6) The pituitaries preserved in absolute ethyl alcohol remain equally potent for over two years as fresh ones.

(7) The frogs kept in captivity for a larger duration show more failure and indifferent results than those freshly collected.

(8) The frogs do not show zoological specificity and can be induced bred by heteroplastic pituitaries.

(9) The pituitaries of dying or just dead frogs also maintain their potency.

A large number of frog in the tropical regions are reported to breed throughout the year (see Chruch, 1960 a,b; Zeller, 1960; Inger and Greenberg, 1963; Berry, 1964; Inger and Baccon, 1968; Brown and Alcala, 1970; and Duellman, 1970). Berk (1930); Bragg (1950 and Ballinsky (1969) have reported that many tropical anurans breed in the nature at any time of the year and at places were temperature and rainfall are sufficiently high. McCann (1933) and Ramaswami and Lakshman (1959) mentioned that the aquatic frog, Rana cyanophlyctis is capable of breeding throughout the year if suitable conditions are provided to them. Thus in the present investigation induced breeding of Rana cyanophlyctis was attempted every month from January to December, and successful response was recorded from March to October. The failure of breeding during November, December, January and February may be attributed to very low atmospheric temperature (Fig. 1.2.3).

Heusser (1961); Gunther (1969); Smith (1969); Wahl (1969) and Van Gelder and Hoedemackers (1971) reported that in warmer regions, a population of frogs, abdding in permanent water bodies, may have several short period of peak breeding activity. Rana cyanophlyctis seems to belong to the same category, and has prolonged breeding season at Shillong, which coincide with the rainy months.

The failure to achieve induced spawning in Rana cyanophlyctis weighing less than 5.8 cm and 25.0 gm, is attributed to its immature age having small immature oocyte which are not competent to respond to the gonadotropin stimulus as also reported earlier by Schuetz (1969), Evennett and Thornton (1971. Gangadhara and Ramiah (1968) observed that starvation in the *Rana cyanophlyctis* destroyed the mature follicle in the ovary. Jorgensen (1976) noted degeneration of non-ovulated egg rapidly due to starvation. Failure in the induced ovulation of the stored frog, during present investigation may be due to the poor feeding in captivity.

Fecundity:

Terentjev (1960) observed linear relationship between fecundity and SV length in certain Russian frogs and subsequently derived a regression equation to calculate fecundity of the available frogs from its know SV length.

$$\log F = -1.7428 + 2.1670 \log L$$

where

F = Fecundity
L = SV length of frog

Inger and Greenberg (1966); Inger and Baccon(1968) noticed that fecundity observed in the natural conditions and fecundity calculated in accordance to Terentjev's regression equation, showed little variation in rain forest frogs. In the present investigation a linear relationship between fecundity and SV length of female frogs have been observed (Fig. 6.2). Further, the bivariate correlation coefficient derived between the two variables described above have also been found to high (r = 0.94 at 19 degree of freedom,'df'). The relationship between fecundity and SV length (Fig. 6.2) in Rana cyanophlyctis is found to be expressed by log F = -1.4169 + 6.0851 log L.

Applying SV length of the Rana cyanophlyctis measured during present investigation to the Terentjev's equation at log base 'e' and log base 'n' the fecundity in the various size group of the frog has been calculated (Table 6.10). The fecundity calculated accordingly showed great variations from the observed and calculated fecundity in the frog derived in accordance to the regression equation calculated earlier for the Rana cyanophlyctis. However, in both the cases of frogs from different populations, the linear relationship and linear regression in bivariates have been observed. The relationship between SV length and weight of the ova has also been derived (Fig.6.3) and was observed to be expressed by the linear regression equation , with fairly high correlation coefficient (r = 0.96 at 19 df.)

$$\log W = -4.506 + 6.4369 \log L$$

Further, significant correlation coefficient and linear relationship between, weight of the frog and weight of ova observed ($r = 0.86$; 19 df.) weight of the frog and the weight of ova calculated ($r = 0.92$; [illegible]) has been observed. [illegible] Koskela and Pasanen (1975) working on Rana temporaria observed a linear regression and high correlation coefficient between (1) SV length of the frog and weight of ova (2) SV length and weight of oviduct; (3) SV length and number of egg; (4) SV length and size of egg($r = 0.8$ to 0.9; $P < 0.01$). They also concluded that the value of spawn size and number of eggs depend on the size of female frog and calculated that Rana temporaria of SVL 78 mm would produce 13.5 to 14.0 gm of spawn, consisting of about 1360 eggs of a size of 2.01 mm. Similarly, with the help of equation derived for Rana cyanophlyctis it is noted that a female measuring 7.15 cm would produce 9.8 gm of spawn comprising of 6048 of size 1.5 mm each. However, aliquots analysis and gravimetric measurement at the same SV length it was observed that it had 8.12 to 11.58 gm of ova, consisting of 4465 to 5808 eggs of an approximate size of 1.5 mm.

Further linear relationship in the bivariates (1) gonado somatic index and weight of ova (2) gonado somatic index and weight of frog (3) gonado somatic index and SV length of frog, have been observed. The correlation coefficient of the three relationship described above have been 0.91 ; 0.71 and 0.84 respectively which is recorded to be significantly high at 1% and 5% probability level. Hence, with the help of these derived equations, ova size, ova weight, ova number can be known at a given SV length or body weight of frog, without sacrificing the animals.

SUMMARY

This chapter deals with induced breeding and gonadosomatic index. Homoplastic pituitaries preserved in absolute ethyl alcohol were used for induced breedings. They were found to retain their potency for a period of 3 years and the frogs responded to them as effectively as to fresh ones. Induced breeding could be performed for 8 months, from March to October. The females having SVL 5.8 cm and above and weight 25.0 gm and above, responded successfully to the pituitary infection. 0.08 mg/gm wt of the female was effective in breeding season. During pre and post breeding period a larger dose 0.15 mg/gm - 0.2 mg/gm wt of the females was found to be effective. The maximum number 667 ova were obtained during May and minimum, 27 ova during March. In some experiments, induced breeding for the second time, was also successfully performed on the same frogs. Significant correlation coefficient ($P < 0.01$) and linear relationship has been obtained between SVL and number of ova, SVL and weight of ova, body weight and number of ova, body weight and size of ova, gonadosomatic index and body weight, gonadosomatic index and SVL; and gonadosomatic index and weight of the ovaries.

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