Studies on certain aspects of ECOLOGY and DEVELOPMENT of *Rana limnocharis* Wiegmann

Debjani Roy Ray

Ph. D. Thesis

DEPARTMENT OF ZOOLOGY
SCHOOL OF LIFE SCIENCES
North-Eastern Hill University
SHILLONG - 793003
MEGHALAYA - INDIA

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Grams : NEHU

Phone : 3390

North-Eastern Hill University

DEPARTMENT OF ZOOLOGY
SCHOOL OF LIFE SCIENCES
SHILLONG - 793003

Dr. M.K. Khare,
M.Sc., D.Phil.
Reader

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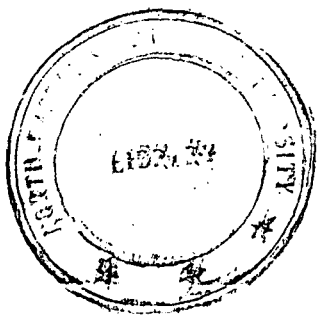
C E R T I F I C A T E

I, the undersigned, certify that this Thesis entitled "STUDIES ON CERTAIN ASPECTS OF ECOLOGY AND DEVELOPMENT OF RANA LIMNOCHARIS WIEGMANN" submitted by Mrs. Debjani Roy for the degree of Doctor of Philosophy of the North-Eastern Hill University, Shillong embodies the record of original investigation carried out by her under my supervision during the period 1975-79. She has been duly registered and the thesis presented is worthy of being considered for the award of Ph.D. degree. This work has not been submitted for any degree to any other University.

M K Khare

(M.K. Khare)
Reader in Zoology.



CONTENTS

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Debjani Roy

(Debjani Roy)

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Department of Zoology,
School of Life Sciences,
North-Eastern Hill University,
Shillong-793003 (Meghalaya),
India.

General Introduction

The group Amphibia comprising of toads, frogs and their congeners, is one of the largest living orders among the vertebrates. There are as many as 2,600 species belonging to 250 genera (Heusser, 1974) and these extend through all temperate and tropical lands except perpetually snow-capped mountains, waterless deserts and some islands of the Pacific. True frogs of the genus Rana Linnæus are found all over North America, Central America, Northern part of South America, Europe, Asia, Africa except its frozen and desert areas and Northern Australia.

For reasons that hardly need enumeration here, frogs have constituted as one of the most favourable animal types for a wide range of biological studies for centuries; and indeed in the current age of standardization in every field, numerous biomedical research projects are being conducted with amphibians that are not standardized for any of the basic characters, such as age, source, nutrition, species, physiology, health or genetics (Nace, 1968) - evidently because of the facility with which these animals are subjected to experimentation.

Many astounding concepts of Developmental Biology to-day have come up as a result of studies on anurans and urodels. Frogs and toads have been used for demonstrating many fundamental concepts in animal physiology, tissue transplantation,

regeneration mechanisms in limb development and in medical studies by gynaecologists for pregnancy tests. And now with an increasing awareness of the value of frogs as food, they are considered as animals of considerable economic importance.

Frogs legs are now served as gourmet dishes in many parts of the world. To ensure the desired supply of frogs for this purpose, however, dependence on nature has to be supplemented with, if not replaced by, frog culture which has thus resumed great significance in recent times.

Priddy and Culley (1971) have reviewed the work on frog culture among recent endeavours. In 1968, at Lousiana State University in the United States of America, a research programme was initiated with N.I.H. which supports to the culture of Rana catesbiana, Shaw, Rana grylio Stejneger and Rana pipiens Schreber. Concurrently at the University of Michigan an amphibian facility was started to culture Rana pipiens and 22 other selected species of amphibians (Nace, 1968). Arizona State University have developed a management system for Ambystoma maxicanum Shaw and some other aquatic amphibians. At the Institute for the study of mind, drugs and behaviour at the Layola University, Illinois, successful maintenance techniques for adult ranids, hylids and bufonids have been developed. In Japan, at the University of Hiroshima, ranids, hylids and discoglossids have been

successfully maintained. Attempts on frog culture have also been made in Italy, Austria and England.

Indian frog-legs have attained a good international market. In the past few years MPEDA (Marine Products Export Development Authority) have been exporting tons of frozen frog-legs. In 1978, they exported 3570 tons worth i.e. Rs. 8,42,51,000.00. Most of this export is dependent upon frogs collected from the natural resources. Besides severely disturbing the ecosystem, this practice would lead to rapid depletion of our frog resources in the country. Therefore, the need of developing efficient frog culture practices cannot be over emphasised. Although successful rearing of ranids, like Rana tigrina Daudin, Rana crassa Jerdon, Rana hexadactyla Lesson and Rana catesbiana Shaw, in frog/fish combination ponds has been reported by Central Inland Fisheries Research Institute, Barrackpore (Mondal, 1975); and work on the care and maintenance of certain Indian frog species has been undertaken at Utkal University (Mohanty-Hejmadi, 1974); we are still far from having evolved reliable methods of frog culture. It needs hardly be stated that for any such attempt in respect of any species, a knowledge of its biology and ecology must be known in detail.

In a preliminary investigation on survey of frogs in 1975-76 (Sahu, unpublished) Rana limnocharis Wiegmann, the

streaked frog, was found to be the commonest species available at Shillong and the neighbouring hills. It is used as a food item among certain tribal communities in the North-Eastern Hill States of India. It has also been reported to be used as food item in Korea (Heusser, 1974). It is with this view in background that investigations were undertaken to evolve suitable breeding and culture techniques of this frog. Thus, the following aspects of the development and ecology of Rana limnocharis relevant in this context, have been investigated in the present work : (1) Morphological characters and annual cycle. (2) Normal Table of development. (3) A 12-month analysis of the food and feeding habits. (4) Pituitary - gonadal cycle comprising the study of cytological changes in the pituitary gland and gonads through an annual cycle. (5) Induced Breeding and (6) Certain ecological aspects such as spawn size, fertility ratio, survival and mortality and influence of limiting temperature on embryonic and post-embryonic development. It is hoped that studies on these aspects on Rana limnocharis Wiegmann will ultimately help in formulating a technology for the maintenance and mass culture of this species.

REFERENCES

Heusser, H.R. (1974). Frogs and toads in Grzimek's Animal Life Encyclopedia. Vol. 5 (Fishes II and Amphibians) pp 357. Van Nostrand and Reinhold Company, New York.

Mohanty-Hejmadi, P. 1974. Amphibian Fauna of Orissa. Prakrati Utkal University Journal. Science. II (1 and 2): 89-97.

Mondal, A.K. 1975. Frog breeding and its propagation in the context of frogleg industry in India. (Paper presented in the short term training course on the improved techniques of frogleg processing and development of allied industries, Calcutta).

Nace, G.W. 1968. The amphibian facility at the University of Michigan. Bioscience. 18: 767-775.

Priddy, J. and D.D. Culley. 1971. The frog culture industry, past and present. Proceedings of the 25th Annual Conference. South Eastern Association of Game and Fish Commissioners. Charleston, South California. 597-601.

Sahu, A.K. 1976. Survey of frogs of Shillong. M.Sc. dissertation.

Plate I - *Rana limnocharis* Wiegmann (female)

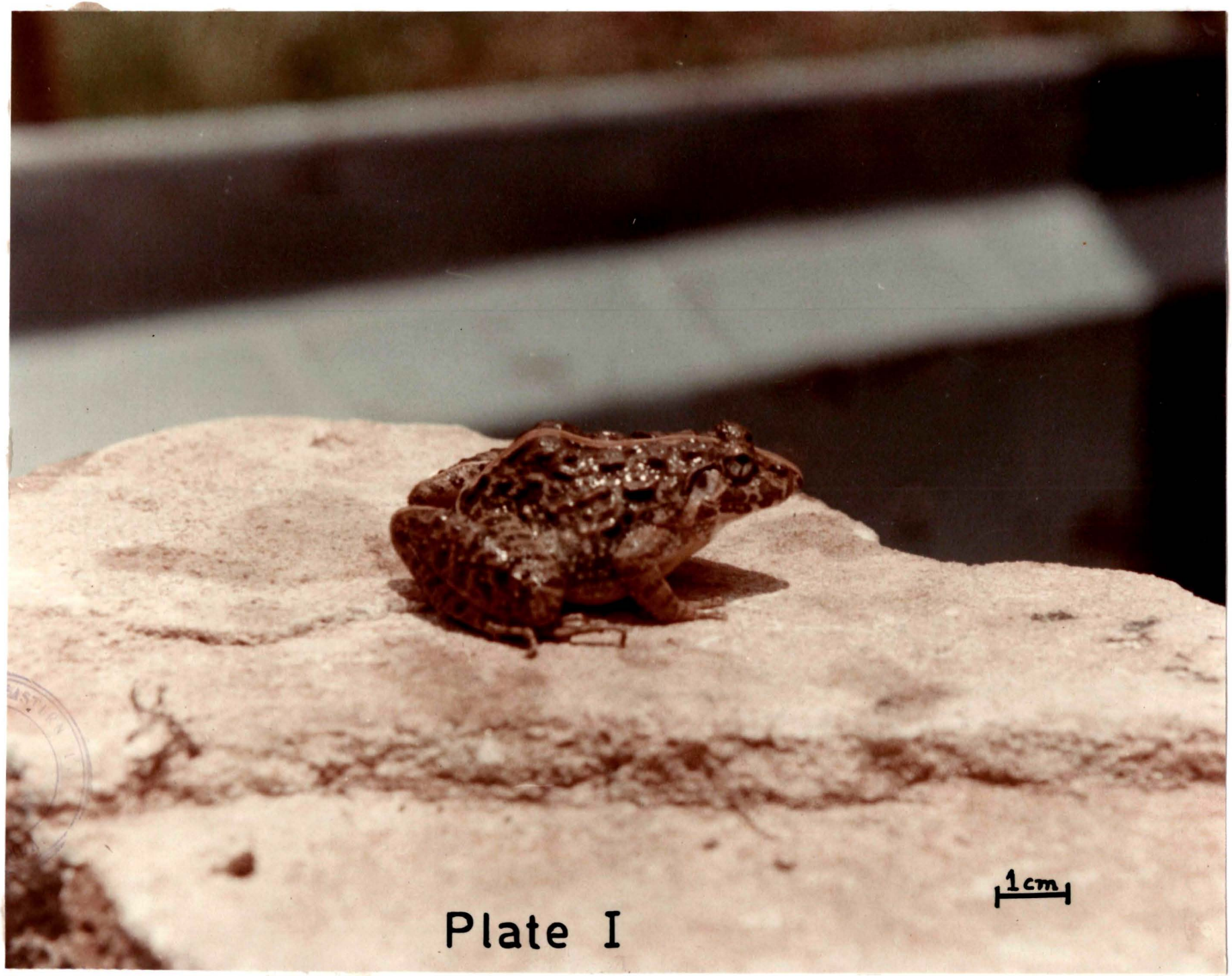


Plate I

Chapter 1

Morphological Characters and Annual Cycle

INTI

Rana limnocharis Wiegmann, the streaked frog (Plate I) is closely related to the common Indian bullfrog, Rana tigrina Daudin, but is much smaller in size and possesses imperfectly webbed toes. The systematic position of Rana limnocharis is

Phylum - Chordata
Subphylum - Gnathostomata
Class - Amphibia
Order - Anura
Suborder - Diplasiocoela
Family - Ranidae
Subfamily - Raninae
Genus - Rana Linnæus
Species - limnocharis Wiegmann

It is one of the widely distributed frog species in India and has been reported from China, Burma, Ceylon, Malay Peninsula, Borneo and Lombok (Satyamurti, 1967). In Himalayas, it has been reported by Acharji and Kirpalani (1951) from Sikkim (2134 metre above sea level) and Kangra and Kulu Valley. It is also found in Nepal, Kumaon and Simla Hills. Boulenger (1920) recorded it from Khasi and Garo Hills in Meghalaya (formerly part of Assam) and other parts of North-Eastern India. As stated earlier it has been found to be the most common species of frog available at Shillong (90°7' E, 24°0' N) at an altitude of 1515 metres in the Khasi Hills. During the active period of its annual cycle

from April to October, it is found mostly at the edges of eutrophic ponds, perennial streams and marshy places. It also occurs in paddy fields in large numbers during paddy season (May-October). Although not very agile, on alarm it goes deep into the water and reappears soon on the surface and swims ashore to seek shelter in the shore vegetation. Many a time it is seen to keep its snout above the surface of the water and whole body hanging below in the water. There is, however, no detailed account of its morphometry and ecology of its high altitude populations.

This chapter has been devoted to the analysis of morphological and taxonomical characteristics and the study of annual cycle of Rana limnocharis found at Shillong. While analysing the taxonomical characteristics, absolute measurements as well as morphometric ratios of various body parts, the criteria adopted by Tinsley (1973 and 1975) has been followed. Investigations on the annual cycle include its breeding activity and population fluctuation in relation to seasonal changes such as temperature and rainfall.

REVIEW OF LITERATURE

A review of literature reveals that workers have mostly concentrated on the study of natural history, taxonomy, distribution, home range movement and population fluctuations

of anurans in relation to environmental changes. Following account deals with a review of contributions on these aspects.

Ever since the publication of the "Biology of Amphibia" by Kingsley Noble (1931) who incorporates early investigations on the systematics and ecology of amphibians, a number of interesting contributions on anuran biology have come up. More important among these are those of Bragg (1941) on the ecology and natural history of anurans of Canadian river flood plain; Bragg and Smith (1942) on breeding behaviour of Oklahoma anurans; Anon (1953) on the ecology of Rana catesbiana and Rana grylio; Zweifel (1955) on the systematics, ecology and distribution of Rana boylei; Savage (1961) on the life history and ecology of Rana temporaria temporaria; Garanin (1961) on the ecology of Rana terrestris in different biotopes; Burkett (1969) on the ecology of cricket frog Acris crepitans; Morris and Tanner (1969) on the ecology of Western spotted frog Rana pretiosa pretiosa; Van Gelder and Oomen (1970) on the ecology of Rana arvalis in The Netherlands and Bloomers-Schlosser and Rose (1975) on certain ecological aspects of development of some Malagasy frogs.

There are a large number of reports on the systematics and taxonomy of anurans but there are very few detailed accounts on their biometry. Berger (1966) furnished a detailed study of the biometry of various parts of Rana esculenta, Rana ridibunda and Rana lessonae. He used 14 various morphometric measurements

and 5 index values in his descriptions and discussed their utility. He also referred to Kauri's (1959) indices showing relationship with environmental parameters like temperature and altitude, but felt that their application was difficult. While studying the biology and systematics of Xenopus and Xenopus vestitus Tinsley (1973 and 1975) has used 9 indices showing ratios between various body parts besides giving 18 absolute measurements. He has compiled data on weight, sex, breeding, gut contents, colour patterns and parasitic infections. Van Dijk (1966) showed the utility of the ratios in the systematic study of anuran larvae.

The distribution of many anuran species of the world has been given in "Amphibians and Reptiles of the World" by Bogert (1954), "Living Amphibians of the World" by Cochran (1967) and Grzimek's Animal Life Encyclopedia by Heusser (1974). The first good description on Reptilia and Batrachia of the Indian subcontinent was given in the Fauna of British India by Boulenger (1890). Other important contributions on the distribution and systematics of Indian anuran have been made by Annandale (1918); Narayan Rao (1923); Smith (1935); Acharji and Kirpalani (1951); Satyamurti (1967); Moorthy (1968); Roonwal (1963); Mansukhani (1970) and Pillai and Chanda (1976). Satyamurti (1967) made a good compilation of the taxonomy and biology of many Indian anurans. The distribution pattern of the anurans of North-Eastern India, has been recently furnished by Pillai and Chanda (1976).

During the last two and half decades several investigations have been made on population ecology of anurans. In this connection the work of Dole (1947, 1965, 1972); Tracy and Dole (1969); Dole and Durant (1974); Martof (1953); Calef (1973) and Schroeder (1976) are worth mentioning. Dole and his group have investigated the home range movements of Hyla cadaverina, spatial relations and home range of natural populations of Rana pipiens, homing and orientation of Bufo americanus to their home site and movement and seasonal activity of Atelopus oxyrhynchus. Martof studied the dispersal movement for homing response of Rana clamitans. Van Dijk studied the habitat and dispersal of certain South African anura. Most of these workers have used toe clipping method for marking the frogs.

Among the work on fluctuations of anuran populations those of Ashby (1969) on Rana temporaria; Van Gelder and Öomen (1970) on Rana arvalis in The Netherlands; Scott and Starrett (1974) on Agalychinis spurrelli in Costa Rica; Heym (1975) on Rana esculenta, Rana ridibunda and Rana lessonae; Zimka (1971) Rana arvalis in Poland; Günther (1974) on Rana ridibunda, Rana lessonae and Rana esculenta in German Democratic Republic and Knoflacher (1975) on a frog population in East Austria deserve mention. They have mostly concentrated on various aspects of population dynamics such as its relation to reproduction, growth and migration, pH, temperature and humidity, rainfall, food and specific biotope. Recently

Plate II A - Area of collection of frogs at Pologround Shillong : A canal.

Plate II B - Area of collection of frogs at Pologround Shillong : Paddy fields.

Plate II C - Area of collection of frogs at Pologround Shillong : A stream.

Plate II

A

B

C

Koskela (1973) and Koskela and Pasanen (1974) have worked out in great detail the annual cycle and behaviour of Rana temporaria in Northern Finland in relation to environmental factors such as temperature, rainfall and diurnal rhythm.

As far as Indian frogs are concerned, a review of literature reveals that although a number of contributions have been made on taxonomy and distribution, very little information is available of their ecology and developmental biology. Although, Rana limnocharis is available throughout eastern tropics, little is known about its biology and ecology of this high altitude populations. Its taxonomy and distribution has been described by Boulenger (1920) and Satyamurti (1967).

MATERIALS AND METHODS

Live specimens of Rana limnocharis were collected from the Polo Ground area in Shillong (Plate II A,B & C). Analysis of morphological characteristics was made according to the criteria followed by Tinsley (1973 and 1975) on a sample of 25 males and 25 females collected during their breeding period. During paddy season they were available in large numbers in paddy fields. After collection the specimens were brought to the laboratory and preserved in 10% formalin. The colour pattern was studied in

live specimens. The weight of the frogs was taken after blotting their body surface with blotting paper. For measurements dividers were used and an accuracy of the order of 0.1 mm was strived at. For the study of the changes in the population structure, the specimens were collected at regular intervals throughout the year, taking one hour as a unit for each collection. The number of males, females and juveniles was recorded after each collection. The observations on the annual cycle were based on the breeding behaviour of the frog in relation to temperature and rainfall.

OBSERVATIONS

1. ANALYSIS OF MORPHOLOGICAL AND TAXONOMICAL CHARACTERISTICS.

Sex and reproductive condition

The 50 specimens studied comprised half males and half females. All the males were mature. Among the 25 females, 5 were spent (20%), 4 did not have ripe ova (16%) and the rest 16 (64%) were mature, containing ripe ova. Many pairs were found in amplexus and spawning was observed during the period of collection.

Size and weight

Body length of males ranged from 3.1 - 4.1 cm, with a mean length of 3.68 cm. In females, it ranged from 3.7 - 5.6 cm, with a mean of 4.53 cm.

The body weight of males ranged from 3.5 - 4.5 gm, with

Fig. 1 - Dorsal view of Rana limnocharis Wiegmann showing vertebral band and longitudinal ridge on the skin.

Fig. 2 - Lateral view of the head region of Rana limnocharis Wiegmann showing skin fold above tympanum.

Fig. 3 - [illegible] figure showing tibio-tarsal articulation in relation to the position of the eye and snout.

Abbreviations:

Vtbnd - Vertebral band	Fd - Skin fold
Lnrdg - Longitudinal ridge	Ey - Eye
Tym - Tympanum	Snt - Snout
H.lmb - Hind limb	Nst - Nostril
Tbtra - Tibio-tarsal articulation	

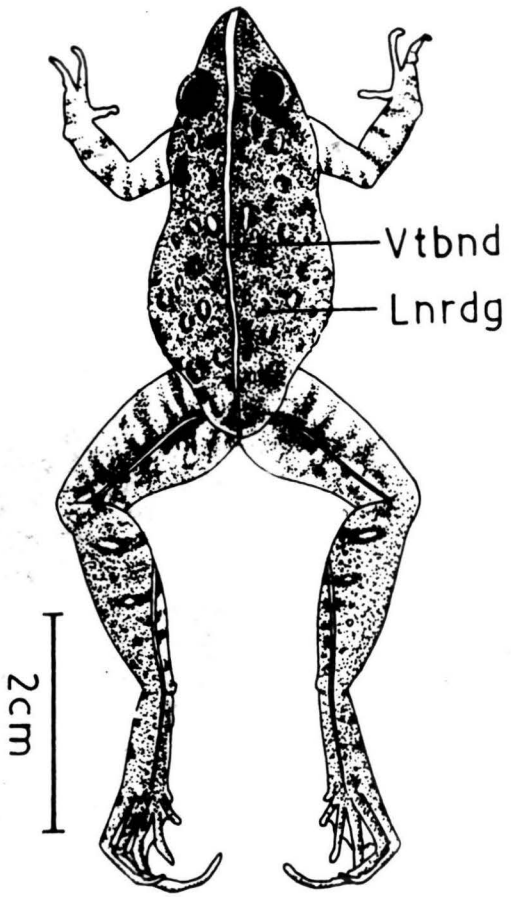


Fig 1

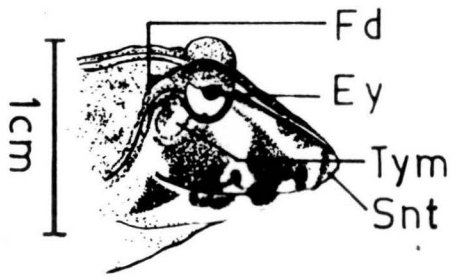


Fig 2

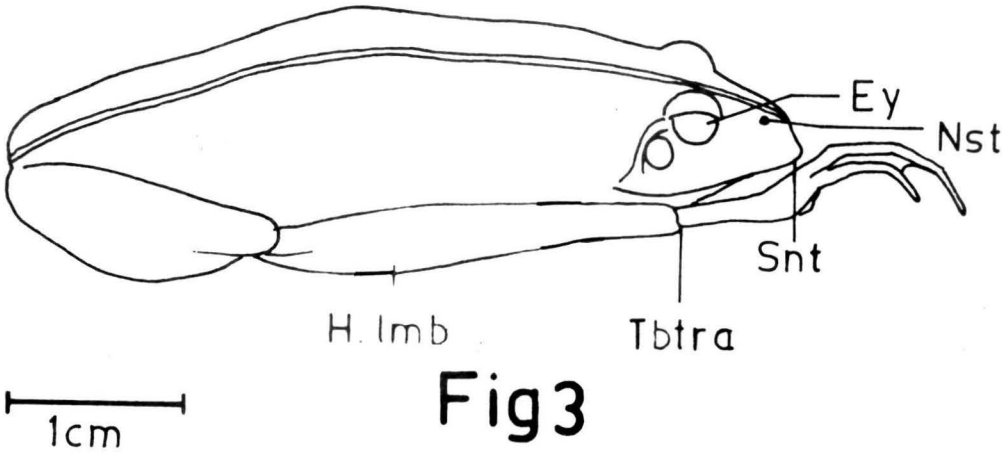


Fig 3

Fig. 4 - Pattern of fingers in the forelimb of Rana limnocharis Wiegmann.

Fig. 5 - Pattern of toes in the hindlimb of Rana limnocharis Wiegmann.

Abbreviations:

Wb - Webb

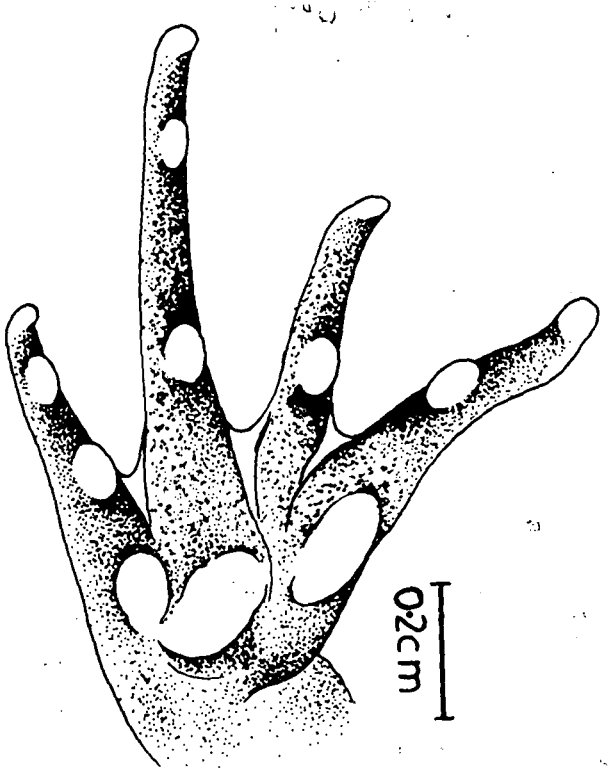


Fig 4

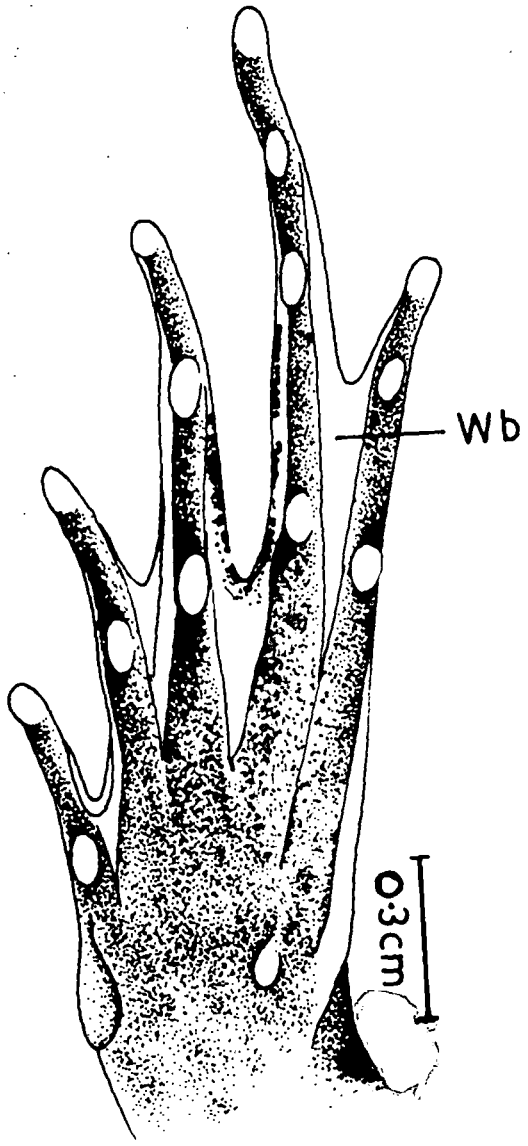


Fig5

a mean weight of 4.06 gm. The weight of females ranged from 8.1 - 13.8 gm, with a mean of 11.72 gm.

Morphological characteristics

1. The skin bears narrow longitudinal ridges on the dorsal side. (Fig. 1).

2. A fold of skin is found above the tympanum and another transverse fold is seen behind each eye. (Fig. 2).

3. Vomerine teeth are arranged in two oblique series between the choane, extending below the level of their posterior border.

4. The head is as long as broad and moderately depressed. (Fig. 2).

5. The snout is pointed or rounded, projecting more or less beyond the mouth. (Fig. 2).

6. The nostrils are situated nearly midway between the eye and tip of the snout, slightly towards the snout. (Fig. 3).

7. The tympanum is distinct and is approximately 1/2 or 2/3 as wide as the eye. (Fig. 2).

8. The fingers and toes are bluntly pointed. (Fig. 4 and 5).

9. The first finger extends beyond the second. The subarticular tubercles are well developed and prominent. (Fig. 4).

10. The hind limbs are moderately long and the tibio-tarsal articulation (heel) reaches the eye or beyond, between the eye and the snout and sometimes even beyond the snout. (Fig. 3).

11. The toes are obtusely pointed and slightly swollen at the end. (Fig. 5).

12. Toes are imperfectly webbed. Fingers are not webbed. (Figs. 4 and 5).

13. There is usually a small outer metatarsal tubercle and a prominent inner metatarsal tubercle.

14. There is a variation in the relative length of the hind limb.

Colour pattern

The dorsal surface is dark brown, or sometimes greyish olive, with large black spots on the back. These black spots are intermingled with yellow and rarely orange markings which are more distinct on the lateral sides of the body. The ventral surface of the body is pale, yellowish white, with dark yellow on the sides bordering the belly wall and the lining of the lower jaw. The lower jaw has narrow black bands on its borders alternating with the yellow colour.

There is a vertebral band on the mid dorsal region varying in colour and thickness, being narrow in some and

broad in others. The colour is yellow, yellowish-orange or creamy white (Plate 1). This band is found to continue on the dorsal aspect of the thigh and inner aspect of the shank upto the foot. Sometimes it is absent in the posterior region of the shank. (Fig. 1).

The limbs have black markings. On the inner surface of the toes and fingers there are black lines with intermediate creamy, white spots at the region of the subarticular joints. The web between the toes is grey and the ventral surface of the toes specially the tips is dark grey.

There are distinct ridges on the back, formed of folds of the skin, which may be black or dark brownish grey. The tympanic region has black round spots surrounded by black ridge on the upper boundary and the lower boundary creamy white.

An analysis of the measurements of the following 15 morphological characters was carried out so as to compare these with the morphometric measurements given by other workers.

1.	Body length	:	Snout to vent.
2.	Body width	:	Measured at the widest point across abdomen.
3.	Head width (minimum)	:	At the tip of the snout, parallel to the nostrils.

4.	Head width (maximum)	:	Measured at the widest point, across the eyes.
5.	Snout length	:	Perpendicular distance from below the nostrils to the tip of the mouth.
6.	Eye diameter	:	Transverse distance across exposed orbits.
7.	Inter-ocular distance	:	Transverse distance between inner bases of circum-orbital plaques.
8.	Nostril diameter	:	Measured across long axis of nostril.
9.	Inter-narial distance	:	Distance between the inner margins of nostril bordering flaps.
10.	Hind limb length	:	Vent to tip of 5th toe.
11.	Tibia length	:	Medial measurement along the dorsal surface of tibia.
12.	5th toe length	:	Measurement of outer ventral surface of the digit.
13.	Total fore limb length	:	Origin of limb to the tip of the 1st finger.
14.	Lower fore limb length	:	Outer angle of elbow to the tip of the 1st finger.
15.	1st finger length	:	Base to the tip of 1st finger.

Measurements of 50 living adult specimens were taken and a summary of data is given in Table I. The relationship

TABLE I

Analysis of Characters of *Rana limnocharis* Wiegmann: 1. Dimensions

Measurements (cm.)

Characters	MALES (25)			FEMALES (25)		
	Mean)	Range	Standard deviation	Mean	Range	Standard deviation
Body length	3.68	3.1 - 4.1	0.21	4.53	3.7 - 5.6	0.48
Body width	1.31	1.1 - 1.8	0.17	1.57	1.1 - 2.1	0.27
Head width (min.)	0.47	0.3 - 0.6	0.07	0.58	0.3 - 0.85	0.12
Head width (max.)	1.17	0.8 - 1.35	0.12	1.28	1.0 - 1.6	0.16
Snout length	0.44	0.3 - 0.85	0.15	0.49	0.3 - 0.8	0.11
Eye diameter	0.57	0.4 - 0.75	0.08	0.60	0.5 - 0.85	0.08
Inter-ocular distance	0.40	0.3 - 0.5	0.07	0.43	0.3 - 0.6	0.07
Nostril width	0.16	0.1 - 0.3	0.07	0.26	0.1 - 0.4	0.07
Inter-narial distance	0.39	0.25- 0.5	0.05	0.51	0.4 - 0.6	0.06
Hind limb length	4.94	4.0 - 5.8	0.57	5.61	5.0 - 6.6	0.38
Tibia length	1.83	1.4 - 2.1	0.17	2.29	1.65- 2.7	0.23
5th toe length	0.68	0.5 - 0.9	0.10	0.76	0.6 - 0.95	0.11
Total fore limb length	2.04	1.4 - 2.7	0.40	2.25	1.9 - 3.1	0.24
Lower fore limb length	0.79	0.55- 1.0	0.11	0.84	0.6 - 1.0	0.11
1st finger length	0.53	0.3 - 0.8	0.16	0.66	0.5 - 1.0	0.10

TABLE - II

Analysis of Characters of *Rana limnocharis* Wiegmann: 2. Ratios

	MALES (25)		FEMALES (25)		OVERALL (25+25)= 50	
	Mean	Range	Mean	Range	Mean	Standard deviation
Head width (min.) / Head width (max.)	0.40	0.27 - 0.50	0.45	0.3 - 0.6	0.425	0.44
Snout length / Head width (min.)	0.94	0.72 - 1.54	0.84	0.6 - 1.0	0.89	0.93
Eye diameter / Inter-ocular distance	1.42	1.0 - 2.17	1.39	0.92 - 1.75	1.40	1.45
Nostril diameter / Inter-narial distance	0.41	0.22 - 1.2	0.51	0.25 - 0.89	0.46	0.51
Tibia length / 5th toe length	2.69	2.22 - 3.6	3.01	1.94 - 4.17	2.85	2.92
1st finger length / Lower fore limb length	0.67	0.33 - 0.89	0.78	0.56 - 1.17	0.72	0.75
Total fore limb length / Body length	0.55	0.38 - 0.74	0.50	0.40 - 0.70	0.525	0.54
Body length / Hind limb length	0.74	0.62 - 0.90	0.81	0.72 - 0.96	0.775	0.79

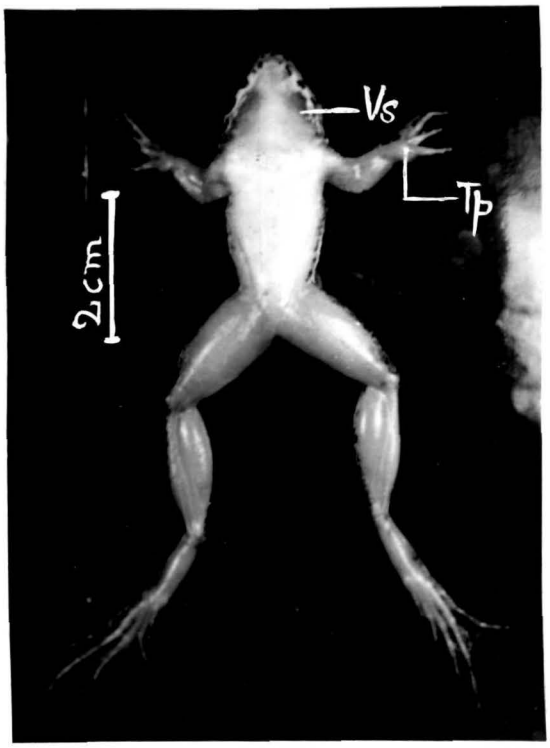
Plate III A - Ventral view of Rana limnocharis Wiegmann (Male)

Plate III B - Ventral view of Rana limnocharis Wiegmann (Female)

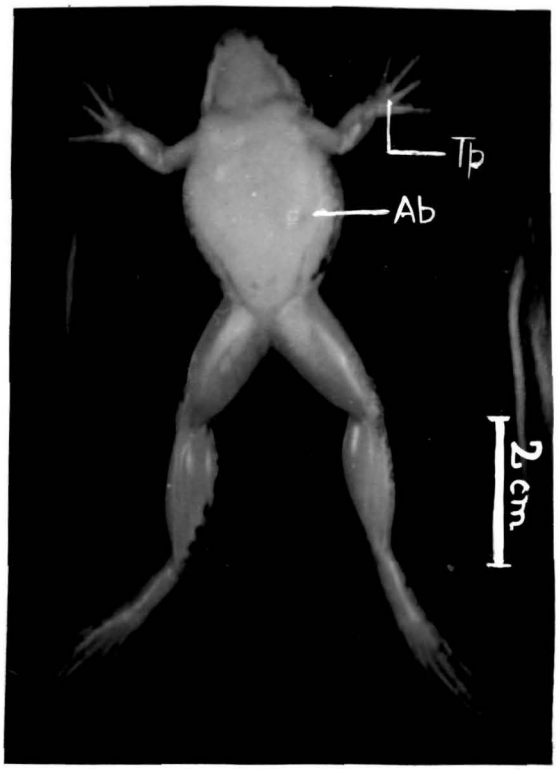
Abbreviations:

Vs - Vocal sac
Tp - Thumb pad
Ab - Abdomen

Plate III



A



B

between 8 of the above features was evaluated to define the distinguishing characters of this species. These ratios are presented in Table II.

Distinguishing characters of male and female of Rana limnocharis.

Besides dimensions and ratios shown in Table I and II, the sexes can be distinguished by the following diagnostic characters :-

MALE (Plate III-A)

1. Mature male frogs are smaller and lighter in comparison to female frogs.

2. It has a relatively slender and streamlined body.

3. It has a prominent thumb-pad which become darker and thicker in the breeding season.

4. It has black markings on the vocal sacs below the throat.

5. In the breeding season, the lateral vocal sacs are swollen and produce a distinct, low to high guttural croaking sound.

FEMALE (Plate III-B)

1. Mature female frogs are larger and much heavier in comparison to the males.

2. Their abdomen is much swollen, depending upon the maturity of the ovaries.

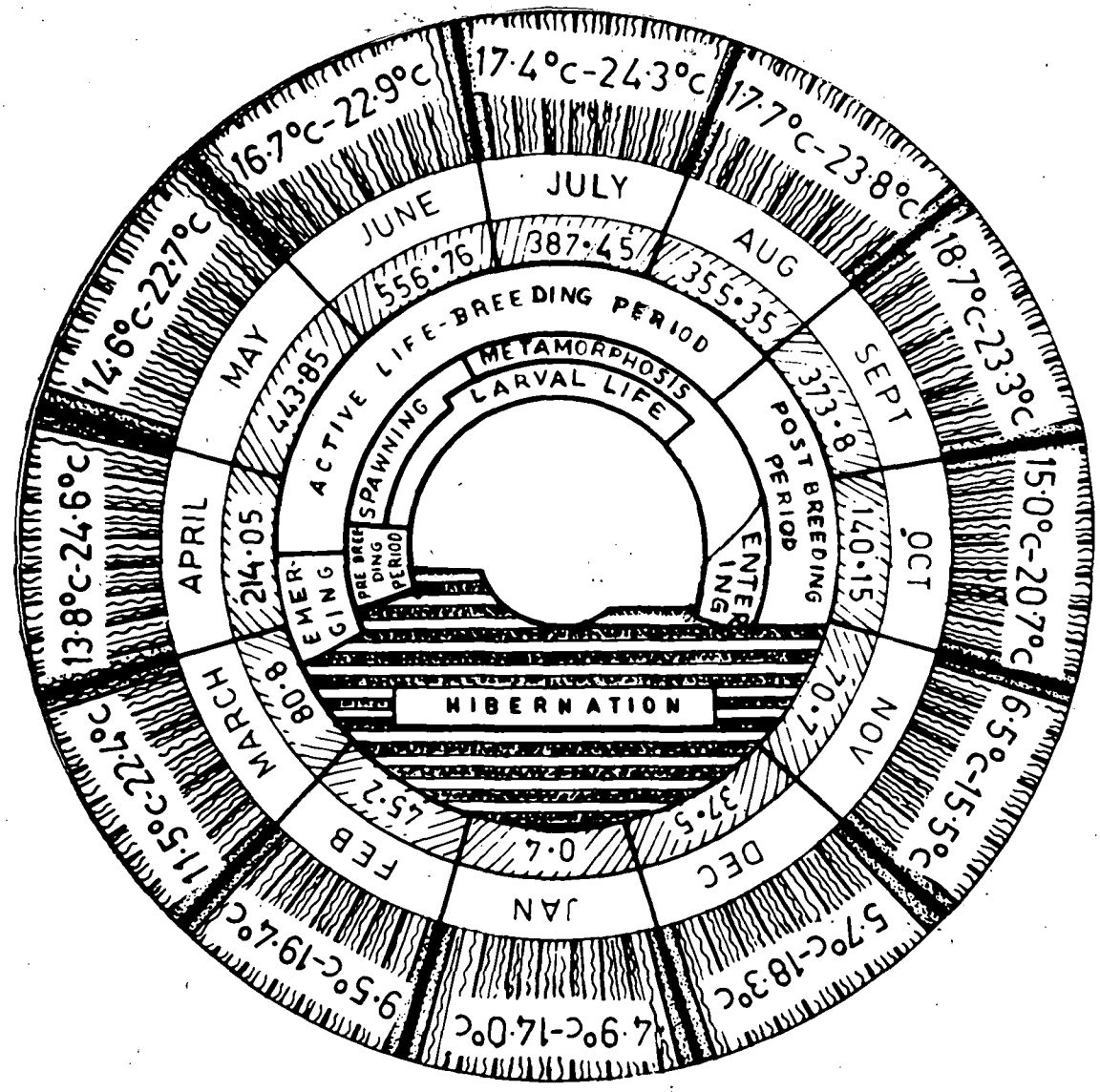
3. The skin of the abdomen in the region of groins is thinner and translucent so that the ripe ova in the abdomen can be seen through it.

4. Females can be identified at any time by the absence of the thumb-pad, absence of lateral cheek pouches and inability to croak.

2. ANNUAL CYCLE.

The annual cycle of Rana limnocharis can be divided into four phases (1) Emerging and 'Pre-breeding period' (2) Spawning and 'Breeding period' (3) Entering and 'Post-breeding period' and (4) 'Hibernation period'. (Fig. 6). The emergence of frogs from the hibernation period is observed in late March when average minimum and maximum temperatures are recorded to be 11.5°C and 22.4°C. This is usually associated with the first showers of rains. The period since the emergence of frogs to the start of amplexus and spawning constitutes the Pre-breeding Period. From the first or second week of April when the minimum and maximum temperatures are recorded to be 13.8°C and 24.6°C and rainfall increases (average 214.05 mm), the frogs show intensive feeding, active movements, amplexing behaviour and start spawning. From this

Fig. 6 - Diagrammatic representation of the annual cycle of Rana limnocharis Wiegmann.



ANNUAL CYCLE OF RANA LIMNOCHARIS WIEGMANN AT SHILLONG

FIG. 6

time to August the frogs lead active life and breed. This period is called the Breeding Period. During breeding period the spawns are seen in temporary ponds, ditches and very slow moving streams often attached to submerged vegetation. Early larval stages are seen from the middle of May when the temperature fluctuates between 14.6°C (min.) and 22.7°C (max.) with an average monthly rainfall of 443.85 mm. The temperature during June is recorded to be 16.7°C (min.) and 22.9°C (max.); during July 17.4°C (min.) and 24.3°C (max.); and during August 17.7°C (min.) and 23.8°C (max.). The rainfall during these months is recorded to be 556.76 mm, 387.45 mm and 355.35 mm respectively. The metamorphosed froglets are observed from early June. Larvae hatched from spawns laid during May and June start metamorphosing by July or August. From June onwards and specially after August large number of young froglets are observed. During middle of October when temperature is reduced to 15.0°C (min.) and 20.7°C (max.) and average monthly rainfall becomes 140.15 mm, the number decreases indicating that they start entering hibernation. The period from September to late October can be designated as Post-breeding Period. The frogs remain in hibernation from November to middle or late March. The average atmospheric temperature during November is noted to be 6.5°C (min.) and 15.5°C (max.); during December 5.7°C (min.) and 18.3°C (max.); during January 4.9°C (min.) and 14.0°C (max.); and during February 9.5°C (min.) and 19.4°C (max.). The

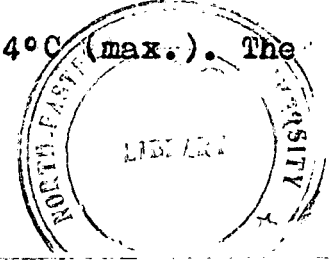


Fig. 7 - Number of frogs collected per unit time during different months of the year in 1976 and 1977.

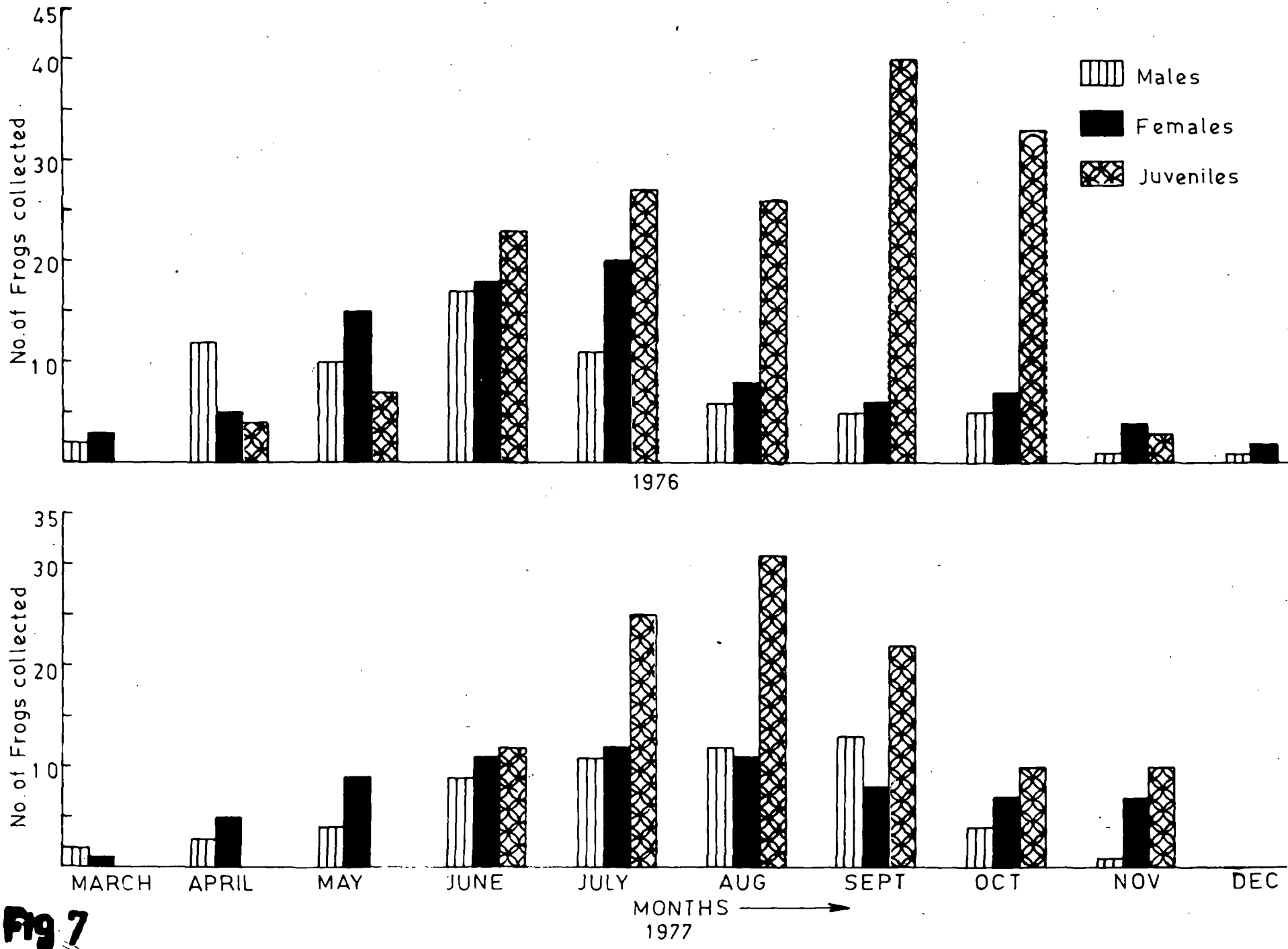

45
40
30
20
10
No.of Frogs collected
Males
Females
Juveniles
1976
35
30
20
10
No.of Frogs collected
MARCH
APRIL
MAY
JUNE
JULY
AUG
SEPT
OCT
NOV
DEC
MONTHS
1977

Fig 7

rainfall during these months is recorded to be 70.7 mm, 37.5 mm, 0.4 mm and 45.2 mm respectively. During hibernation the frogs are found in burrows, sometimes below stones and rarely outside in the vicinity of ponds.

The population structurewas analysed from the samples collected at two regular intervals each month for one hour every time during 1976 and 1977. The data for each month has been illustrated in Fig. 7. The frogs were easily collected from April to October. Minimum number was caught in March and November. It was very difficult to find them during December, January and February. During both the years the number of frogs collected went on increasing from April to July/August and then showed a gradual decline. From June onwards until the start of hibernation, the number of juveniles collected were much more every month. The sex ratio during 1976, collection was found to be approximately 9 females for 7 males. In 1977 it was found to be approximately 7 females for 5 males. The females were always more than the males, in each collection

DISCUSSION

MORPHOMETRIC AND ECOLOGICAL CONSIDERATIONS.

Rana limnocharis is a widely distributed species throughout the eastern tropics; it is found in warmer plains as well as subtropical high altitudes (Satyamurti, 1967). Studies on the systematics of anurans are based mostly on

on specific morphological characters. Tinsley (1973 and 1975), while studying the systematics of Xenopus and Xenopus vestitus, reported that there may be intraspecific variations depending upon the ecology of a species and described many such variations. There are no such studies available on the frog species found in India and neighbouring countries. It was with this aim that besides the description of the specific morphological characters, fifteen absolute measurements, viz., body length (snout vent length or SVL), body width, head width (min.), head width (max.), snout length, eye diameter, interocular distance, nostril width, internarial distance, hind limb distance, tibia length, 5th toe length, total fore limb length, lower fore limb length, first finger length and eight ratios, viz., Head width (min.)/Head width (max.), Snout length/Head width (min.), Eye diameter/Interocular distance, Nostril diameter/Internarial distance, Tibia length/5th toe length, 1st finger length/Lower fore limb length, Total fore limb length/Body length, Body length/Hind limb length, have been analysed in the present investigation (Tables I and II) as per the criteria followed by Tinsley. The general morphological character of Rana limnocharis available at Shillong do not show any important variation in comparison to the descriptions available in the literature (Boulenger, 1920). The vertebral band is said to be absent in some cases (personal

communication, Zoological Survey of India, Eastern Regional Station, Shillong) but none of the specimens were observed in the present investigation without such bands. Satyamurti (1967) has given body length, lengths of fore limb and hind limb of Rana limnocharis collected from seven different localities in the South India. Most of them were young ones. The SVL of full grown specimen from Yercaud was 53 mm. Boulenger (1920) gave SVL of full grown specimens from various places in South-East Asia. They were : Japan : males 40 mm; Loo Choolds : males 48/49 mm and females 50 mm; Sanghai : females 39-46 mm; Formosa : females 46 mm; Hongkong: females 40-45 mm; Sikkin : males 43 mm, females 45-64 mm; Darjeeling : female 39 mm; Nilgiri : males 43 mm, females 45-64 mm; Malabar : females 45-47 mm; Trivandrum : females 33 mm; Madras : females 35-37 mm; Ceylon : females 52 mm; Bangkok : males 44/45 mm and females 51 mm; Siam : females 46 mm; Malacca : females 57-67 mm; Singapore : males 38 mm and females 57-60 mm; Java : males 46 mm and females 42-50 mm; Lombok : females 42-50 mm; and Borneo : females 56-62 mm. In the present investigation on Rana limnocharis the SVL of males was found to be 36 mm and of the females 45 mm. The weight of males ranged from 3.5 to 4.5 gm and that of females from 8.1 to 13.8 gm during breeding season. This supports that there may be intraspecific variations. The utility of ratios of various body parts has been increasingly felt in the systematic analysis by recent workers (Berger, 1966;

Van Dijk, 1966 and 1977; Tinsley, 1973 and 1975). Berger (1966) quotes Terentiev (1950) who while arguing on the utility of the ratios said, "as far as a definition of differences between various forms is concerned various well chosen ratios of different body parts provide much greater informative value". The utility of these ratios has further been elaborated by Van Dijk (1966) in the systematic studies of anuran larvae. Absolute measurements may vary but ratios between various body parts remain almost constant. This has been tested in different anuran tadpoles in our laboratory also (Mr. A.K. Sahu, personal communication). Tinsley (1975) has also taken into consideration the weight, sex, breeding behaviour, gut contents, colour pattern and parasitic infections in his systematic analysis of Xenopus. It is felt that these informations may be relevant to ecological interactions and may account for adaptation and distribution of different species. Investigations on all aspects except parasitic infections of Rana limnocharis have been included in the present thesis, but a detailed study of all these aspects and morphometric ratios of various populations of this species from different localities would be required to understand the ecological interactions in its adaptation and distribution.

ANNUAL CYCLE

Systematic records of the annual cycles of many frog species found in India are still not available. A detailed

account of the life history and developmental table of Rana tigrina from North India has been recently given by Agarwal and Niazi (1977). Fragmentary information is available on the life cycles of Rana crassa, Rana hexadactyla and Rana cyanophlyctis. Life cycles of many anurans found in North America has been compiled by Rugh (1962). A detailed representation of the annual cycle of Rana temporaria has been recently given by Koskela (1975). There are few reports available on Rana limnocharis. Boulenger (1920) reported that they undergo prolonged hibernation in mountainous districts. Satyamurti (1967) described that populations of Rana limnocharis in South India spawn at the onset of monsoon and aestivate during dry season under stones or damp places. The present investigation is the first 'complete' record of the annual cycle of high altitude population of Rana limnocharis. It has been divided in four distinct periods : (1) 'Emerging' and 'Prebreeding' (2) Spawning and 'Breeding' (3)Entering and 'Postbreeding' and (4) 'Hibernation periods'. Prebreeding period is a very short period, since emergence from the hibernation in late March and the breeding period starts in April. Emergence of the frogs from hibernation is usually associated with the first shower of rain. By the first week of April the frogs enter the breeding period. Mating cells are heard, they go into amplexus and spawn. Breeding period continues till August. The postbreeding period lasts during September

and October when the frogs do not spawn, juvenile stages are seen in large numbers. From November to late March the frogs live in hibernation. The emergence of frogs from hibernation and start of breeding period is clearly associated with increased temperature and first shower of rains. The onset of hibernation is associated clearly with low temperature and reduced rainfall. Increase in the number of the frog population during active period is associated with the increase in temperature and high rainfall.

SUMMARY

This chapter deals with an analysis of morphological characters and morphometric ratios and study of the annual breeding cycle of the streaked frog, Rana limnocharis Wiegmann found in Shillong and the neighbouring areas. The size (snout-vent length) of the males ranged from 3.1 to 4.1 cm and that of females from 3.7 to 5.6 cm. The body weight of the males ranged from 3.5 to 4.5 gm and that of the females from 8.1 to 13.8 gm. While analysing the morphological characteristics, 15 absolute measurements along with 8 morphometric ratios of various body parts were taken into consideration, since morphometric ratios are known to be constant for a given species. The males were identified by small slender body, presence of vocal sacs and thumb pads during the breeding season; whereas females lacked these characteristics and had larger bodies with swollen abdomen.

The annual cycle has been divided in four periods: Pre-breeding, Breeding, Post-breeding and Hibernation periods. With the atmospheric temperature ranging from 11.3 C to 22.2 C with an average rainfall of about 80.8 mm during the second fortnight in March, the frogs come out of hibernation and entered the pre-breeding period. By the middle of April with temperature ranging from 13.8 C to 24.6 C and the average monthly rainfall being 214.05 mm, the frogs entered into a period of peak breeding activity. The spawning commenced with

the first shower of rains in April and the young metamorphosed froglet stages were commonly seen by June. By the middle of October when the temperature fluctuated between 15°C and 20.7°C with a rainfall of 140.1 mm, the frogs once again entered into hibernation. As a result of it during December and January which are the coldest months in Shillong not even a single specimen could be seen in the open natural habitat.

The regular monthly sample collections from the field showed that the population was maximum during breeding period; and the number of the females was more than the males in most of the collections. Juveniles were more from June onwards, often much higher in number than the adults.

REFERENCES

Acharji, M.N. and M.B. Kirpal Ani. 1951. On a collection of reptilia and batrachia from the Kangra and Kulu valleys, Western Himalayas. Rec. Ind. Mus. XLIX. : 175-184.

Annandale, N. 1918. Chelonia and Batrachia of Inle Lake. Rec. Ind. Mus. XIV. pp. 67.

* Anon. 1953. The ecology of Rana catesbiana and Rana grylio. Carolina Tips. 16.

Ashby, K.R. 1969. The population ecology of a self-maintaining colony of a common frog (Rana temporaria). J. Zool. Lond. 158: 453-474.

Berger, L. 1966. Biometrical studies on the population of green frogs from the environs of Poznan. Ann. Zool. (Warszawa) 23(11): 303-324.

Bloommers-Schlosser and M.A. Rose. 1975. Observations on the larval development of some Malagasy frogs with notes on their ecology and biology (Anura: Dyscophinae, Scaphiophryninae and Cophylinae). Beaufortia 24(309): 7-26.

Bogert, C.M. 1954. Amphibians and Reptiles of the world in Drimmer. Donbleday and Co. New York.

Boulenger, G.A. 1920. A monograph of the South Asia, Papuan, Melanesia and Australian Frogs of the genus Rana. Rec. Ind. Mus. XX: 28-33.

Bragg, A.N. 1941. Observations on the ecology and natural history of Anura. XI The invasion of the Canadian River. Flood Plain by two prairie species. Proc. Oklar. Acad. Sci. 22: 73-75.

Bragg, A.N. and C.C. Smith. 1942. Observations on the ecology and natural history of Anura. IX Notes on the breeding behaviour in Oklahoma. Gt. Basin. Nat. Prov. Utah. 3(2): 32-50.

Burkett, R.D. 1969. The ecological study of the cricket frog, Acris crepitans in North Eastern Kansas. Diss. Abstr. Int. 30B: 2956-2957.

Calef, G.W. 1973. Spatial distribution and "effective" breeding population of red-legged frogs (Rana aurora) in Marion Lake, British Columbia. Can. Field. Nat. 87(3): 279-284.

Cochran, D.M. 1967. Living amphibians of the world. Doubleday and Co. Inc. New York.

Dole, J.M. 1947. Home range in the canyon tree frog. (Hyla cadaverina). The South Western Naturalist. 19 (1): 105-119.

Dole, J.M. 1965. Spatial relations in natural populations of the leopard frog, Rana pipiens Schreber in Northern Michigan. Am. Midl. Nat. 74(2): 464-478.

Dole, J.M. 1972. Homing and orientation of displaced toads, Bufo americanus to their home sites. Copeia.1: 151-158.

Dole, J.M. and P. Durant. 1974. Movements and seasonal activity of Atelopus oxyrhynchus (Anura : Atelopodidae) in a Venezuelan cloud forest. Copeia. 1: 230-235.

* Garanin, V.I. 1961. [Ecology of the sharpnosed frog (Rana terrestris)] Izvest. Kazansk. Filiala. Akad. Nauk. SSR. Ser. Obshch. 1: 196-199.

Guenther, R. 1974. New data on the distribution and ecology of the frogs (Anura, Ranidae) of the German Democratic Republic. Mitt. Zool. Mus. Berl. 50(2): 287-298.

Heusser, H.R. 1974. Frogs and toads in Grzimek's Animal Life Encyclopedia.5 (Fishes II and Amphibian). Van Nostrand and Reinhold Co. New York.

Heym, W.D. 1975. Studies on the distribution, ecology and ethology of the frogs of the Central and Northern Niederlausitz region. Mitt. Zool. Mus. Berl. 50(2): 263-285.

* Kauri, H. 1959. Die Rassenbildung bei europaischen Rana - Arten und die Gultigkeit der Klimaregeln. Ann. Soc. Tart. Invest. Const. Ser. nov. Lund. 2: 1-174.

* Knoflacher, H.M. 1975. Production studies on a frog population of the Neusiedler Lake. Oesterr. Akad. Wiss. Math-Naturwiss K L Sitzungsber ABT I. 184 (8-10): 369-378.

Koskela, P. 1973. Duration of the larval stage, growth and migration in Rana temporaria L. in two ponds in Northern Finland in relation to environmental factors. Ann. Zool. Fennici., 10: 414-418.

Koskela, P. and S. Pasanen. 1974. The wintering of the common frog Rana temporaria L., in the Northern Finland. Aquilo. Ser. Zool. 15: 1-17.

Mansukhani, M.R. 1970. Fauna of Rajasthan, India. VI. Amphibia. Rec. Zool. Surv. India 62(1/2): 51-60.

Martof, B. 1953. Home range and movements of the green frog, Rana clamitans. Ecology. 34: 529-543.

Morris, R.L. and W.W. Tanner. 1969. The ecology of the Western spotted frog, Rana pretiosa pretiosa Baird and Girand, a life history study. Great Basin Natur. 29: 45-81.

Moorthy, T.S.N. 1968. On a collection of amphibians from Madras and its neighbourhood. J. Univ. Poona. Sci. Technol. 34: 63-71.

Narayan Rao, C.R. 1923. Notes on a collection of Batrachia from South Waziristan. J. Bomb. Nat. Hist. Soc. XXIX : pp 131.

Noble, K. 1931. The biology of amphibia. Dover publications, New York.

Pillai, R.S. and S.K. Chanda. 1976. Distribution pattern of amphibia in North East India. J. Assam. Sci. Soc. 19(1): 53-56.

Roonwal, M.L. 1963. Fauna of Rajasthan, India. I : General introduction, with a list of collecting localities and a bibliography of Rajasthan zoology. Rec. Zool. Surv. India. 61 (3/4): 291-376.

Satyamurti, S.T. 1967. Bulletin of the Madras Government Museum. Natural History Section. VII (2). Government of Madras Publication.

Savage, R.M. 1961. The ecology and the life history of the common frog. (Rana temporaria temporaria) London : Pitman.

Schroeder, E.E. 1976. Dispersal and movement of newly transformed green frogs, Rana clamitans. Am. Midl. Nat. 95(2): 471-474.

Scott, N.J. Jr. and A. Starrett. 1974. An unusual breeding aggregation of frogs with notes on ecology of Agalychinis spurrelli. Bulletin of the South California Academy of Sciences. 73(2): 86-94.

Smith, M.A. 1935. The amphibians and reptiles obtained by Capt. Kingdom Ward in Upper Burma, Assam and South West Tibet. Rec. Ind. Mus. 37: 237-240.

Tinsley, R.C. 1973. Studies on the ecology and systematics of a new species of clawed toad, the genus Xenopus from Western Uganda. J. Zool. Lond. 169: 1-27.

Tinsley, R.C. 1975. The morphology and distribution of Xenopus vestitus (Anura : Pipidae) in Central Africa. J. Zool. Lond. 175: 473-492.

Tracy, C.R. and J.M. Dole. 1969. Orientation of displaced California toads, Bufo boreas to their breeding sites. Copeia. 4: 693-700.

Van Dijk, D.E. 1966. Systematic and field keys to the families, genera and described species of South African Anuran tadpoles. Ann. Natal. Mus. 18(2): 231-286.

Van Dijk, D.E. 1977. Habitats and dispersal of Southern African anura. Zoologica Africana. 12(1): 169-181.

Van Gelder, J.J. and H.C.J. Öomen. 1970. Ecological observations on the amphibia in The Netherlands. I. Rana arvalis Nilsson. Reproduction, growth, migration and population fluctuation. Neth. J. Zool. 20(2): 238-252.

* Zimka, J.R. 1971. Analysis of the change in the density of frogs (Rana arvalis, Nilss.) under varying conditions of humidity and food resources in forest habitats. Bull. Acad. Pol. Sci. Ser. Sci. Biol. 19(7/8): 479-484.

* Zweifel, R.C. 1955. Ecology, distribution and systematics of frogs of the Rana boylei group. Univ. California. Publ. Zool., 54: 207-292.

* Not consulted in original.

Chapter 2

** Normal Table of Development

** Paper published:

D. Roy, D and M.K.Khare. 1978. Normal Table of De
of Rana limnocha ris Wiegmann. Proc. Nat.
India 48 (B):5-16.

INTRODUCTION

The study of development of anurans is considered basic to the training of embryologists world over, and a table of developmental stages of these has been found to be the foremost necessity. Various developmental tables of anurans described by different developmental biologists have been included in practical laboratory manuals such as Hamberger (1960) and Rugh (1962). Recent laboratory manuals, such as New (1966) and Billet (1975) have included the developmental table of Xenopus laevis only, as it has become one of the common laboratory animals in most of the western laboratories. They have described these developmental stages after Nieuwkoop and Faber (1967). Nieuwkoop and Faber have also compared the developmental tables of various anuran species. In 1960, Gosner gave a simplified table for staging anuran embryos and larvae with notes on identification. Despite so many developmental tables, there appears to be some variation in the description of later stages of development. Based on external criteria the most exhaustive tables appear to be on Rana pipiens by Shumway (1940) on prefeeding period and by Taylor and Kallros (1946) on postfeeding period. Somewhat similar pattern of description has been followed by Agarwal and Niazi (1977) in their normal table of developmental stages of an Indian frog species Rana tigrina. Significant developmental events form a basis while describing the normal tables of development in

urodela. Each new developmental event characterizes a developmental stage. Seeing the utility of stages in the experimental work the criteria for staging followed by Nieuwkoop and Faber (1967) has been found to be most convincing as it incorporates both external and internal morphological changes. None of the Indian species studied so far from the developmental point of view have been studied after induced breeding. The normal table of development of Rana limnocharis Wiegmann, in the present investigation, has been prepared after induced breeding. An earlier developmental table on Rana limnocharis Boie found in Singapore described by Hock and Wen (1970) includes the description only upto late tadpole stage. In the present investigation, complete development from unfertilized egg to froglet stage has been studied and described into 32 stages, each based on a significant external morphological change. The changes in the external morphology as described by Nieuwkoop and Faber (1967) have been adopted as the main criteria in this description.

REVIEW OF LITERATURE

The need for normal table of development of anurans appears to have been realised perhaps as early as their importance for academic pursuits in late 19th century. Normal tables of development have been described for different species of anurans dividing the development into a number of

stages each based on a notable morphological change. Thus, Alder (1901) while describing the development of Bufo vulgaris divided the whole development in 15 stages. Pollister and Moore (1937) described the development of Rana sylvatica in 25 stages up to limb bud stage. Shumway (1940) described the development of Rana pipiens into 25 stages up to complete operculum stage of the tadpole. In 1942, he identified the stages with the help of sectioned material. Eakin (1946) while studying the determination and polarization of regularity in the retina of Hyla regilla, described its developmental stages also. In 1946, Taylor and Kollros described the post-embryonic development of Rana pipiens larvae from Shumway stage 25 and divided the larval stages into another 25 stages. In 1957, Gallien and Houillon described the development of Discoglossus pictus in 32 stages up to tadpole stage, when limb bud starts appearing. In 1954, Michniewska-Predygier and Pigon described the early developmental stages of Rana temporaria, Rana terrestris, Rana esculenta and Bufo bufo. Tahara (1959) described the normal developmental stage of Rana japonica. Kopsch (1952) described 30 developmental stages of Rana fusca up to metamorphosis. During the same year, Del Conte and Sirlin described the development of Bufo arenarum into 25 stages when operculum is completed in the tadpole. Cambar and Marrot (1954) described the development of Rana dalmatina in 54 stages upto metamorphosis. Cambar and Gipouloux (1956)

gave a chronological table of embryonic and larval development of Bufo bufo. In 1959, Cambar and Martin described the development of Alytes obstetricans. In 1960, Gosner gave a simplified table for staging anuran embryos and larvae, with notes on identification. Many developmental tables have been described for Xenopus laevis (Peter, 1931; Gasche, 1944; Bretscher, 1949 and Nieuwkoop and Faber, 1956). A resume of all these descriptions has been given in"Normal table of development of Xenopus laevis" by Nieuwkoop and Faber (1967). They have given a most exhaustive developmental table ever described. They have divided the development up to metamorphosis into tadpole stage in 66 stages, based on external as well as internal anatomical features. As Xenopus laevis has proved to be a very favourable experimental material in many international laboratories, these stages have proved to be of great help in performing transplantation experiments at different stages. Khan (1965) has described the developmental table of Bufo melanostictus into 43 stages up to metamorphosis. Hock and Wen (1970) described 25 developmental stages of Rana limnocharis Boie up to tadpole stage from Singapore, but following the criteria of Shumway (1940). Among Indian frogs, a normal table of Rana cyanophlyctis has been described by Ramaswami and Lakshman (1959) covering description only up to tadpole stage. Among other tables available, Limbaugh and Volpe (1957) on the development of Bufo valliceps, Hing (1959) described the developmental stages of Rana chalconata. Jorquera and Izquierdo (1964) on

the development of Rana chilena. Sedra and Michael (1961) on the development of Bufo regularis and Siboulet (1970) on the development of Bufo mauretanicus.

MATERIALS AND METHODS

Live specimens of Rana limnocharis were collected from the Polo Ground area, during early part of the breeding period in late March and April, 1976 and 1977. Egg laying was induced in the laboratory by injecting the gravid females with homoplastic pituitaries preserved in absolute ethyl alcohol at a dosage of 0.1 mg per gm of the body weight. (Dosage were determined separately - See Chapter VI). After about 24 hours, egg masses from the females were stripped directly into the sperm suspension prepared by macerating the testis obtained from mature males in 100 cc of 10% Holtfreter's solution. Batches of each 20-50 eggs were kept in 200 cc of culture medium. After hatching some algae and water plants were added to the culture medium for food. For metamorphosis the tadpoles were transferred to aquaria, set with steep sand base immersed up to a certain level with pond water. This arrangement helps the tadpoles to metamorphose, similarly as in the natural environment. The laboratory temperature at which the development was studied ranged from 11.3°C at night to 20.5°C during day time. Diagrams at each stage were made with the help of camera lucida. Photomicrographs were taken for embryonic stages only.

OBSERVATIONS

Morphological changes were observed on the surface of the egg soon after fertilization. Initiation of cleavage is seen after about 1½ hours. The larva hatched on 5th or sometimes on 6th day. The metamorphosis was completed by 33rd to 36th day. In the following account, the development has been described in 32 stages each based on a significant external morphological change.

STAGE 1: Age 0 Hours; Length 1.46 mm.

Egg at the time of fertilization. One cell stage. Spherical in shape. Pigmentation darker ventrally than dorsally. Egg diameter 1.46 mm. (Fig. 1).

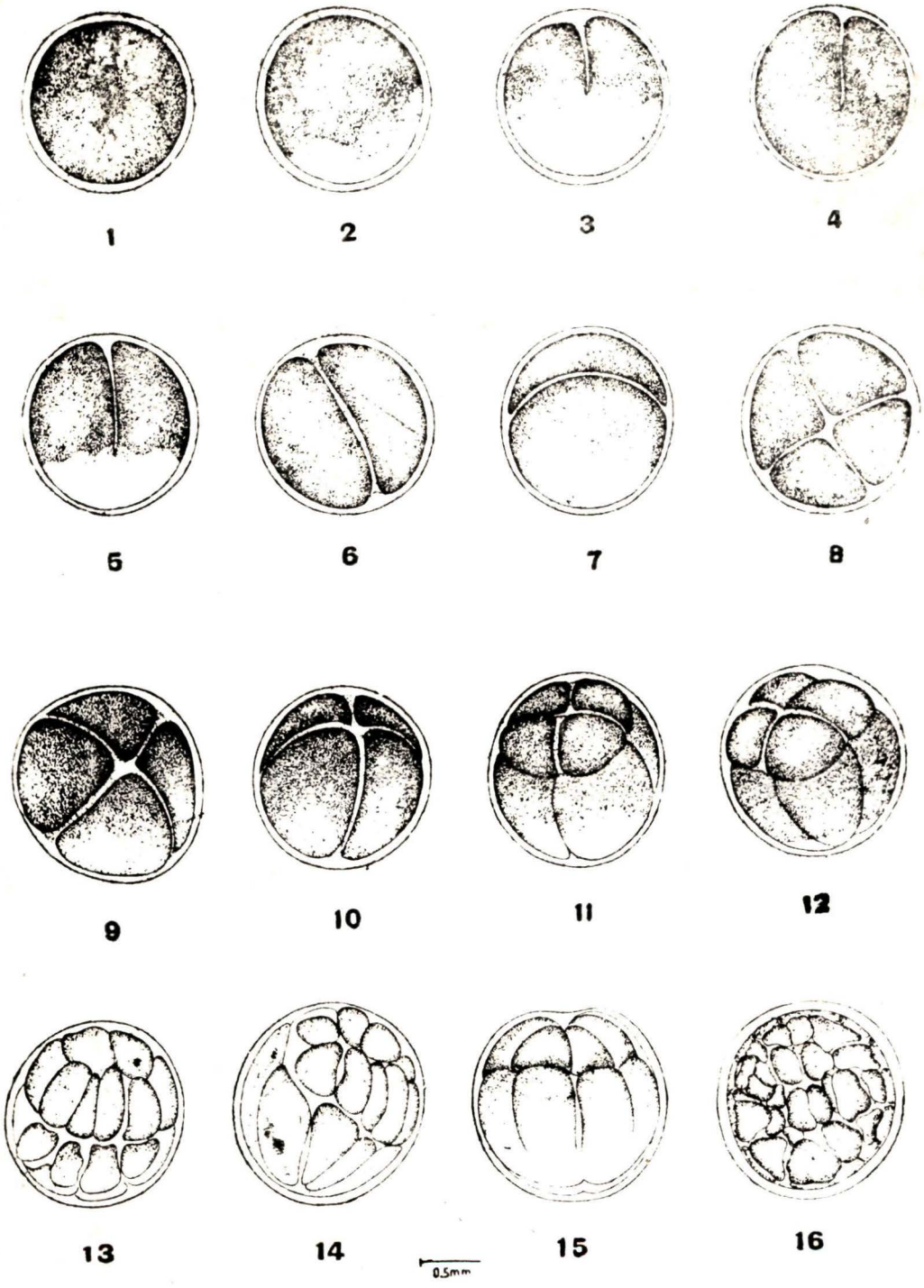
STAGE 2: Age 1½ - 2 Hours; Length 1.46 mm.

"One cell stage" after fertilization. Spherical in shape. Dark pigmentation at animal pole, light pigmentation at vegetative pole. Egg diameter 1.46 mm. Jelly layer distinct with thickness of 0.08 mm. Dark pigmentation extending upto 0.86 mm towards vegetative pole. Light pigmentation 0.43 mm. towards the equatorial region. In between the two pigmentation zones there is a region of medium pigmentation of 0.17 mm (Fig. 2).

STAGE 3: Age 2 - 2½ Hours; Length 1.46 mm.

"Two cell stage" The diameter of the embryo (egg has

Fig. 1	Stage 1	Unfertilized egg.
Fig. 2	Stage 2	Fertilized egg.
Figs. 3-7	Stage 3	First cleavage showing two-cells (different views).
Figs. 8-10	Stage 4	Second cleavage showing four-cells (different views).
Figs. 11-12	Stage 5	Third cleavage showing eight-cells (different views).
Figs. 13-15	Stage 6	Fourth cleavage showing sixteen-cells (different views).
Fig. 16	Stage 7	Fifth cleavage showing thirtytwo-cells.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now become an embryo), jelly thickness and measurement of pigmented areas is almost same as in Stage 2. (Plate I—A; Fig. 3-7).

STAGE 4: Age 2½ - 3 Hours; Length 1.5 mm.

"Four cell stage". The cleavage furrow starts by 2½ hours from animal pole reaches the vegetative pole by 3 hours. Embryonic diameter increased 1.5 mm. Measurement of dark pigment same as in Stage 2, the medium pigment increased over light pigment, the former 0.43 mm. latter 0.21 mm. Jelly thickness same as Stage 2. (Plate I—B,C,; Figs. 8-10).

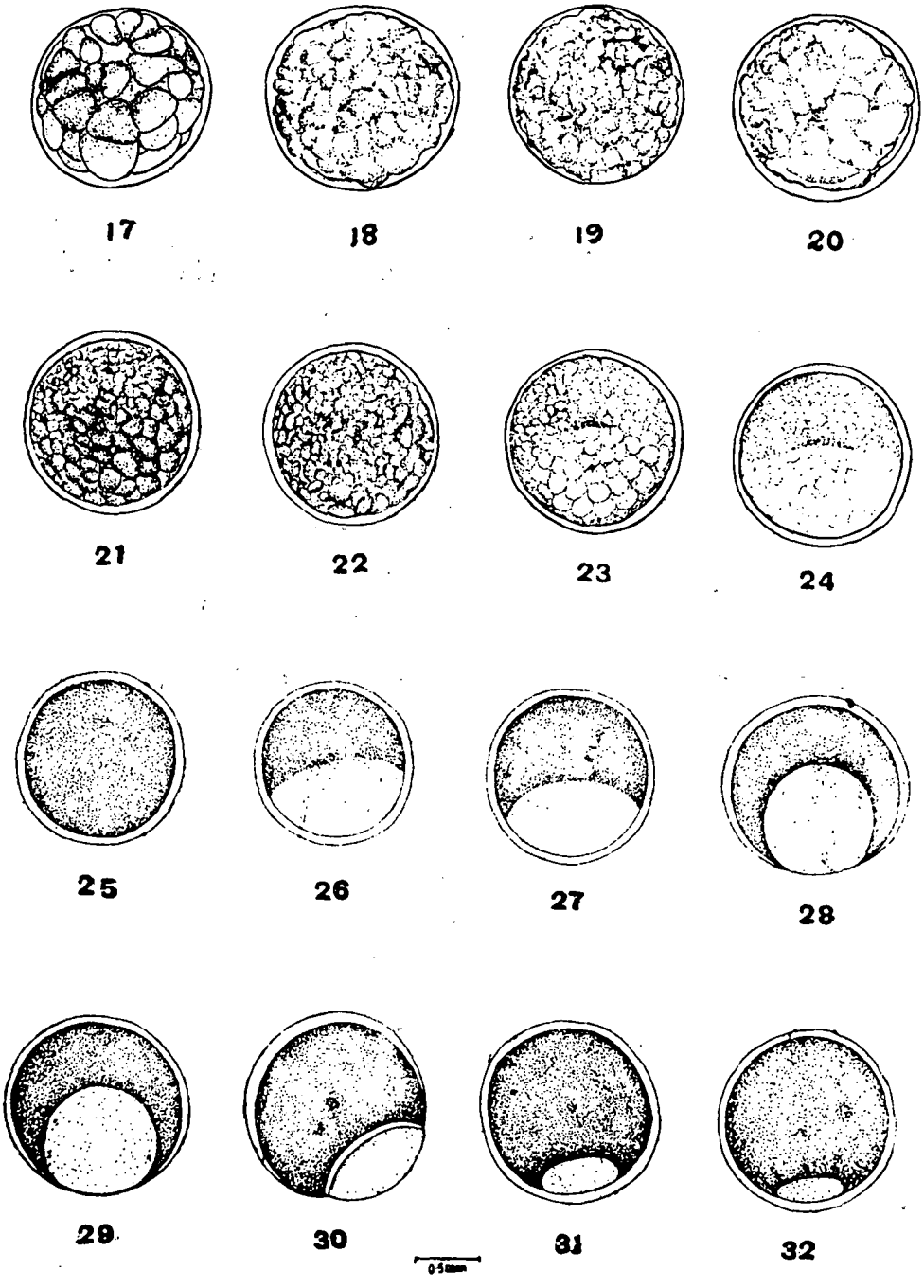
STAGE 5: Age 3 - 3½ Hours; Length 1.5 mm.

"Eight cell stage". The cleavage furrows start by 3 hour and are completed by 3½ hour. Four upper micromeres, smaller and darker; four lower macromeres, larger and lighter. Macromeres have all three types of pigmentation, dark, medium and light. The embryonic diameter and pigment measurement same as at Stage 4 and jelly thickness same as at Stage 2. (Plate I—D; and Figs. 11 and 12).

STAGE 6: Age 3½ - 4 Hours; Length approximately 1.5 mm.

"Sixteen cell stage". The upper dark micromeres are first divided into eight cells, resulting in an intermediate twelve cell stage. By another 4 hours the cleavage furrow reach the vegetative pole, dividing the four lower, light, larger micromeres. The diameter of the embryo, pigment measurement same as Stage 4, jelly thickness same as Stage 2. (Plate II-F; Figs. 13-15).

Fig. 17	Stage 7	Fifth cleavage showing thirtytwo-cells (different view).
Figs. 18-20	Stage 8	Sixtyfour cells (different views).
Figs. 21-22	Stage 9	Late cleavage (different views).
Fig. 23	Stage 10	Dorsal lip formed.
Figs. 24-29	Stage 11	Active gastrulation (different phases).
Figs. 30-32	Stage 12	Yolk plug visible (different phases).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STAGE 7: Age 4 - 4½ Hour; Length approximately 1.5 mm.

"Thirty two cell stage". Embryonic diameter, pigment measurement remaining same as Stage 4, jelly thickness same as Stage 2. (Plate I—G and H; Figs. 16 and 17).

STAGE 8: Age 4½ - 5¼ Hour; Length approximately 1.5mm.

"Sixty four cell or mid cleavage stage" also called "Large cell blastula stage". From this stage there is a clear distinction between the micromeres and macromeres due to gradual transition of cell size from animal to gegetative pole. All measurements almost similar as at Stage 4. (Figs. 18-20).

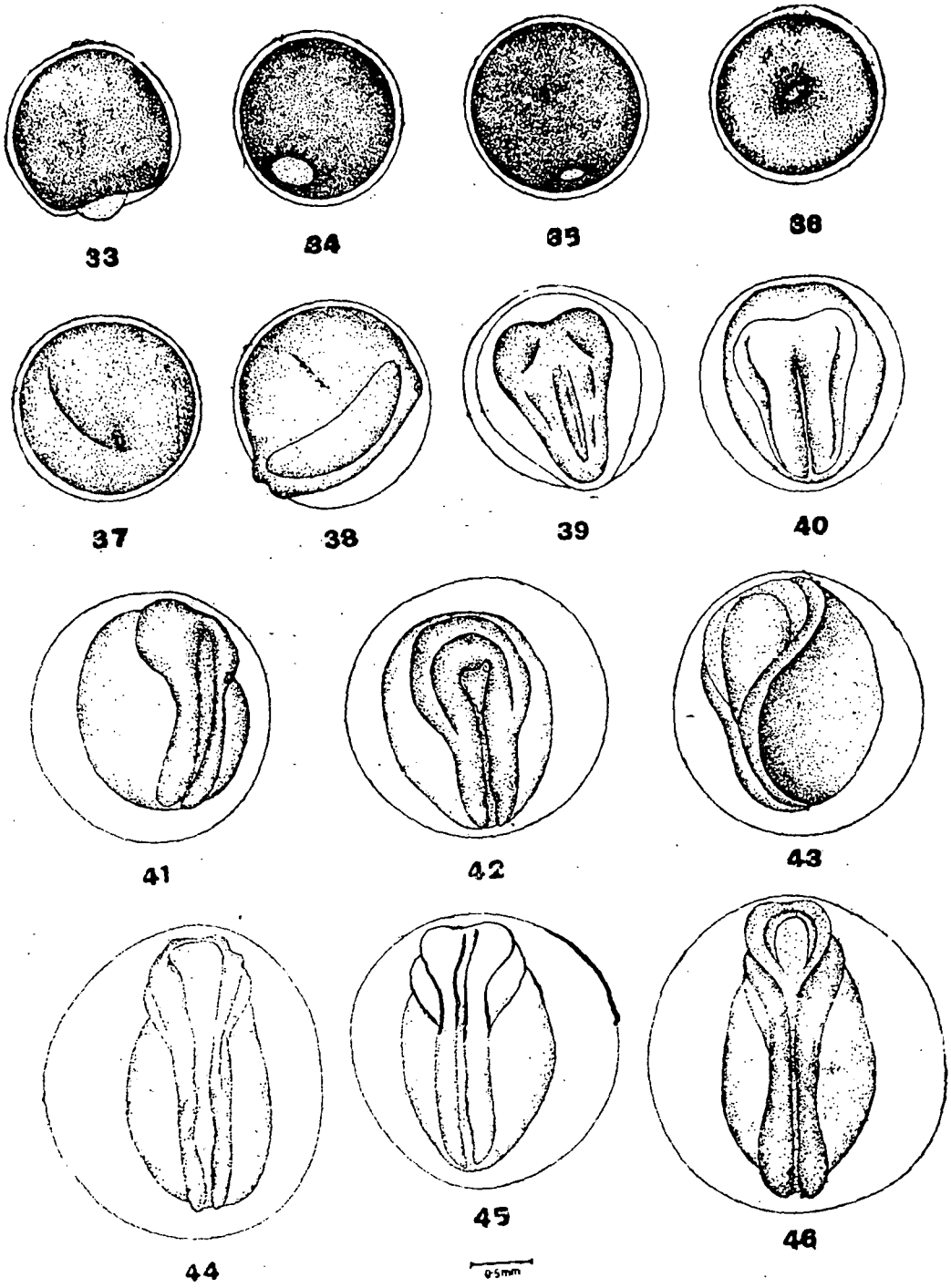
STAGE 9: Age 5¼ - 12½ Hour; Length 1.54 mm.

"Many cell or late cleavage stage" also called "Fine-cell blastula stage". Surface of embryo not smooth by the early hour of this stage. The distinction between micromeres disappeared. Embryo darkly pigmented at animal pole and dark pigment encroached over the area of medium pigment. The diameter of the embryo increased, 1.54 mm. Jelly thickness almost similar as Stage 2. Measurement of dark pigment increased being 1.16 mm, light pigment 0.21 mm and medium pigment 0.17 mm. By 10 hours the cells undergo epiboly, the whole egg surface smooth and no distinction between cell boundaries (Plate I—I; Figs. 21 and 22).

STAGE 10: Age 12½ - 13 Hour; Length 1.59 mm.

"Initial gastrula stage". Appearance of dorsal lip by

Figs. 33-34	Stage 12	Yolk plug visible (different phases).
Figs. 35-37	Stage 13	Disappearing yolk plug (different phases).
Figs. 38-40	Stage 14	Neural plate formed (different phases).
Figs. 41-44	Stage 15	Neural fold appeared (different phases).
Figs. 45-46	Stage 16	Neural groove formed (different phases).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pigment concentration. Embryonic diameter 1.59 mm. Measurement of dark, medium and light pigment being 0.99 mm, 0.17 mm and 0.43 mm respectively. Jelly thickness remaining similar as at Stage 2. (Plate I—J and K; Fig. 23).

STAGE 11: Age 13 - 18½ Hour; Length 1.59 mm.

"Blastopore stage". Active gastrulation indicated by the formation of blastopore. At this site cells invaginate into the interior of the embryo. The growing margins of the blastopore, assume crescent, horse shoe and ultimately circular shape. By pigment concentration it indicates the border of future yolk plug, the egg diameter remaining similar as at Stage 10 and jelly thickness same as Stage 2. (Figs. 24-29).

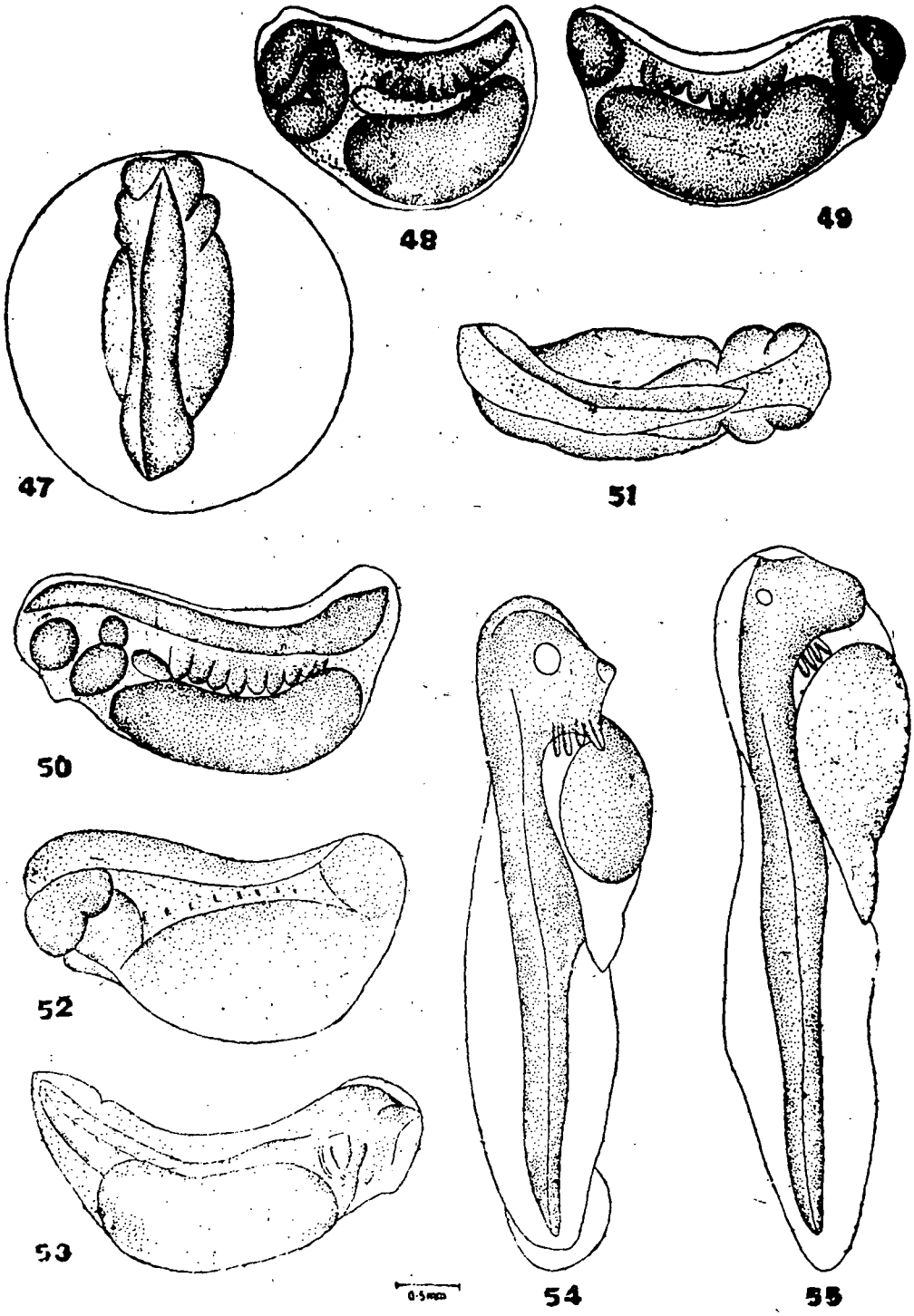
STAGE 12: Age 18½ - 23 Hour; Length approximately 1.59 mm.

"Yolk plug stage". Blastopore groove closed ventrally. Yolk plug large, circular having a diameter of 0.64 mm. the diameter of the embryo same as Stage 10. Jelly thickness same. The yolk plug with light pigment and the rest of the embryo, having dark pigment measuring 1.07 mm. (Plate I—L; Figs. 30-34).

STAGE 13: Age 23 - 30 Hour; Length approximately 1.59 mm.

"Disappearing yolk plug stage". Yolk plug circular, small, more or less pigmented fields radiating from yolk plug. Future position of the neural plate and groove indicated by dark pigment lines. The diameter of the embryo remaining same as Stage 10. Jelly thickness similar as at Stage 2. (Plate II-M,N,O,P,Q; Figs. 35-37).

Fig. 47	Stage 17	Tail bud visible.
Figs. 48-49	Stage 18	Muscular response observed.
Fig. 50	Stage 19	Heart beat observed.
Fig. 51	Stage 20	Gill circulation starts.
Fig. 52	Stage 21	Mouth open.
Fig. 53	Stage 22	Tail fin circulation observed.
Fig. 54	Stage 23	Operculum fold formed.
Fig. 55	Stage 24	Opercular fold closed on right.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STAGE 14: Age 30 - 32 Hour; Length approximately 1.7 mm.

"Neural plate stage". Neural plate delimited. Embryo elongated, measuring 1.7 mm. Jelly thickness remaining similar as at Stage 2. (Plate IV-R; Figs. 38-40).

STAGE 15: Age 32 - 35 Hour; Length approximately 1.72-2.06 mm.

"Neural fold stage". The neural plate distinct and lighter in colour, than the rest of the embryo. The neural folds gradually approach each other from blastopore to anterior region. Jelly distinct. (Plate IV-S and T; Figs.41-44).

STAGE 16: Age 35 - 44 Hour; Length 2.06 - 2.36 mm.

"Neural groove stage". Neural folds touching each other. Considerable extension of the anterior part of the neural plate. Due to the elongation of the embryo, jelly layer distinct, having diameter of 2.36 to 2.59 mm. Lateral outline of the embryo convex. (Plate IV-U; Figs. 45 and 46).

STAGE 17: Age 44 - 50 Hour; Length 2.36 - 2.70 mm.

"Tail bud stage". Delimitation of the anterior region by pigmented lines. Initial motor reactions to external stimulation. Jelly diameter 2.59 - 2.66 mm. Lateral outline of the embryo flat. (Plate IV-V and W; Fig. 47).

STAGE 18: Age 50 - 58 Hour; Length 2.70 - 3.01 mm.

"Muscular response stage". Distinct protrusion of eye. Initial groove between jaw and gill area. Myotomes showing through for the first time. Vitellene membrane becoming wider. Jelly diameter 2.66 to 3.26 mm. (Figs. 48 and 49).

STAGE 19: Age 58 - 68 Hour; Length 3.01 - 3.22 mm.

"Heart beat stage". Beginning of tail fin formation. Myotomes more clearly distinct. For the first time clear separation between head and trunk regions. Tail not yet separated from the trunk. Jelly diameter 3.26 to 3.31 mm. (Fig. 50).

STAGE 20: Age 68 - 91 Hour; Length 3.22 - 3.35 mm.

"Gill circulation stage". Mouth region distinct. Distinction between jaw and gill area. Rudimentary protruberance and grooves at the gill area. Eye spot clear. Fin area broadened near the caudal end of the trunk. Tail fin divided into outer transparent and inner translucent band with muscles. Jelly diameter 3.31 to 3.46 mm. (Fig. 51).

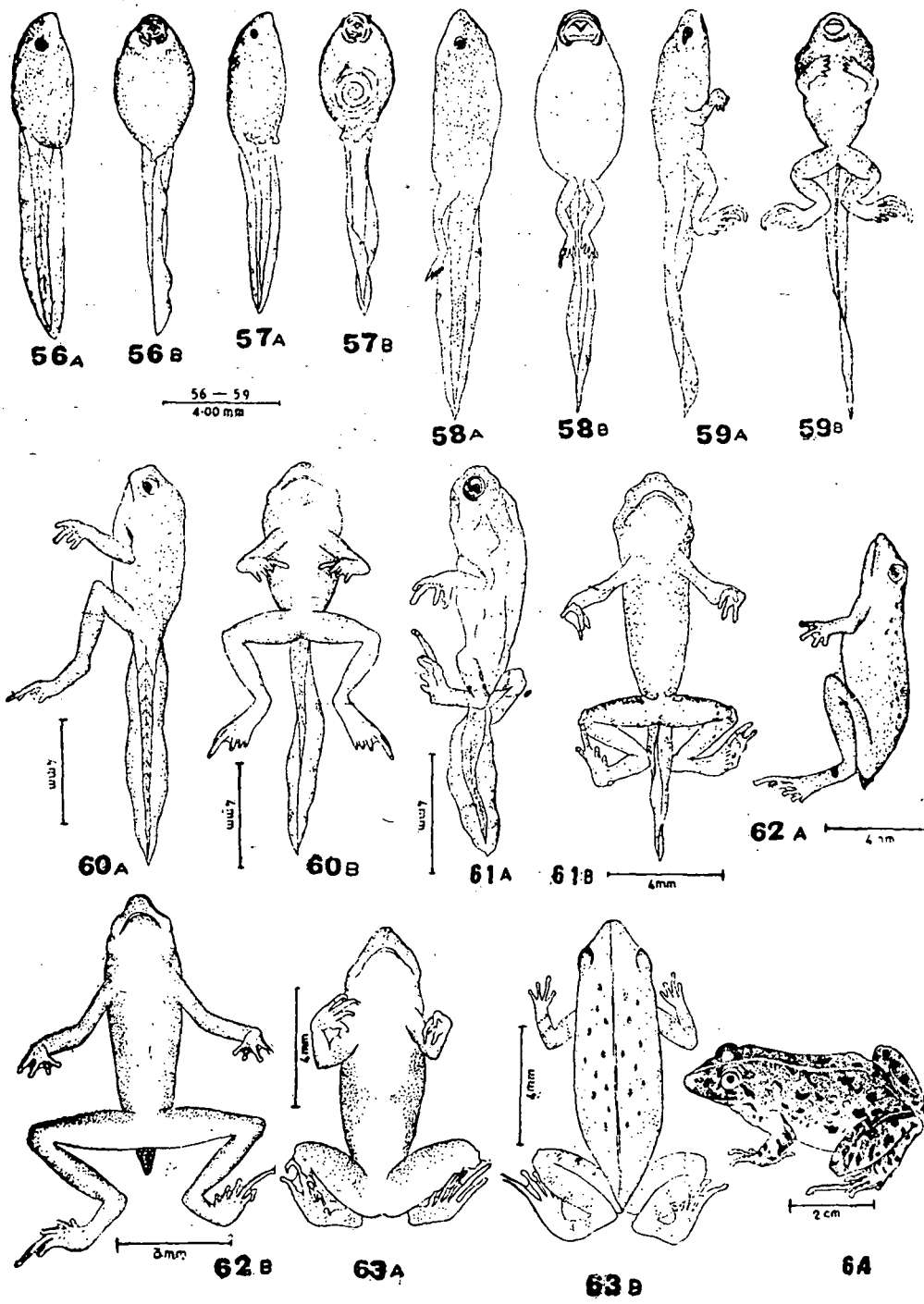
STAGE 21: Age 91 - 115 Hour; Length 3.35 - 5.16 mm.

"Mouth open stage". Mouth distinct with rows of keratodonts and oral papillae. Gills distinct and nipple shaped. Eyes clear, black with light coloured pupil in the middle. Embryo with distinct head about 0.86 mm. long, trunk about 4.30 mm. long. The embryo keeps its body folded inside vitelline membrane (PlateIIW), as it measures more than the jelly diameter. Jelly diameter measures about 4.12 mm. At the end of Stage 21 hatching occurs. (Fig. 52).

STAGE 22: Age 115 - 190 Hour; Length 5.16 - 6.23 mm.

"Tail fin circulation stage". Distinct head about 1.07 mm. long, with eye, ear, mouth and gills. Trunk measuring 2.15 mm., with gut and anal opening clearly visible. Tail, about 3.01 mm. long, with broad fins and tail muscles. (Fig. 53).

Fig. 56 A & B	Stage 25	Operculum closed and complete.
Fig. 57 A & B	Stage 26	Hind limb bud appeared.
Fig. 58 A & B	Stage 27	Hind limb developed.
Fig. 59 A & B	Stage 28	Forelimb sprung.
Fig. 60 A & B	Stage 29	Both limbs developed.
Fig. 61 A & B	Stage 30	Beginning of metamorphosis.
Fig. 62 A & B	Stage 31	Tail stub remaining.
Fig. 63 A & B	Stage 32	Young frog.
Fig. 64		Adult frog.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STAGE 23: Age 190 - 228 Hour; Length 6.23 - 6.45 mm.

"Opercular fold stage". All the characters remaining same as above. At this stage the opercular fold becomes visible for the first time, but it is not complete. Gills projecting below operculum. Gill length shorter than earlier stage. Embryo grown in length, head about 0.84 mm., trunk about 2.36 mm. and tail about 3.44 mm. (Fig. 54).

STAGE 24: Age 228 - 254 Hour; Length 6.45 - 7.51 mm.

Embryo showing its opercular fold closed on right side. All the characters similar as at Stage 23, only the operculum closed on right side of the body. Left gills still can be seen but smaller than before. Head length about 0.84 mm, trunk length about 3.65 mm. and tail length about 4.21 mm. (Fig. 55).

STAGE 25: Age 254 - 288 Hour; Length 7.15 - 10.32 mm.

Embryo having operculum also called "Complete operculum stage". Embryo with only trunk and tail. Distinction between head and trunk disappeared. Operculum closed and external gills disappeared. Eye with well developed lens and retina. Appearance of melanophores and distinct pigmentation pattern. Coiled intestine and tadpole starts feeding on filamentous algae. Tail long, with tail fin and myotomes. Head - trunk length about 3.44 mm. and tail length about 6.88 mm. (Fig. 56 A and B).

STAGE 26: Age 288 - 336 Hour; Length 10.32 - 11.18 mm.

"Hind limb bud stage". Appearance of hind limb bud at the groove between the base of the tail and belly wall. It appears as small white bud like structure. Oral sucker elevation completely disappeared. Two rows of postoral labial teeth present. Eye prominent. Lateral line system present but not distinct. Tadpole having clear and equal pigmentation and not spotted, as in Stage 25. Head - Trunk length about 3.87 mm. and tail length about 7.31 mm. (Fig. 57 A and B).

STAGE 27: Age 336 - 470 Hour; Length 11.18 - 19.55 mm.

"Tadpole with developed hind limb". Pigment free patches appear at the metatotorsophalangeal joints, where the proximal toe pad will later develop. Cloacal tail piece reduced. Fore-limb not yet seen. Head - Trunk length about 7.55 mm. and tail length about 12.00 mm. (Fig. 58 A and B).

STAGE 28: Age 470 - 674 Hour; Length 19.55 - 21.51 mm.

Tadpole with forelimbs just sprung from under the operculum. Larval mouth furnishings absent, by the shedding of the horny beak and labial fringe. Hind limb well developed, used for movement. Head - Trunk length about 7.51 mm. and tail length about 14.00 mm. (Fig. 59 A and B).

STAGE 29: Age 674 - 722 Hour; Length 21.51 - 18.00 mm.

Tadpole with both limbs developed. From this stage tadpole undergoes metamorphosis. Angle of the mouth reached

Plate I A - First cleavage showing two-cells (Stage 3).

Plate I B and C - Second cleavage showing four-cells (Stage 4).

Plate I D - Third cleavage showing eight-cells (Stage 5).

Plate I E and F - Fourth cleavage showing sixteen-cells (Stage 6).

Plate I G and H - Fifth cleavage showing thirtytwo-cells (Stage 7).

Plate I I - Late cleavage (Stage 9).

Plate I J and K - Dorsal lip (Stage 10).

Plate I L - Yolk plug (Stage 12).

Plate I

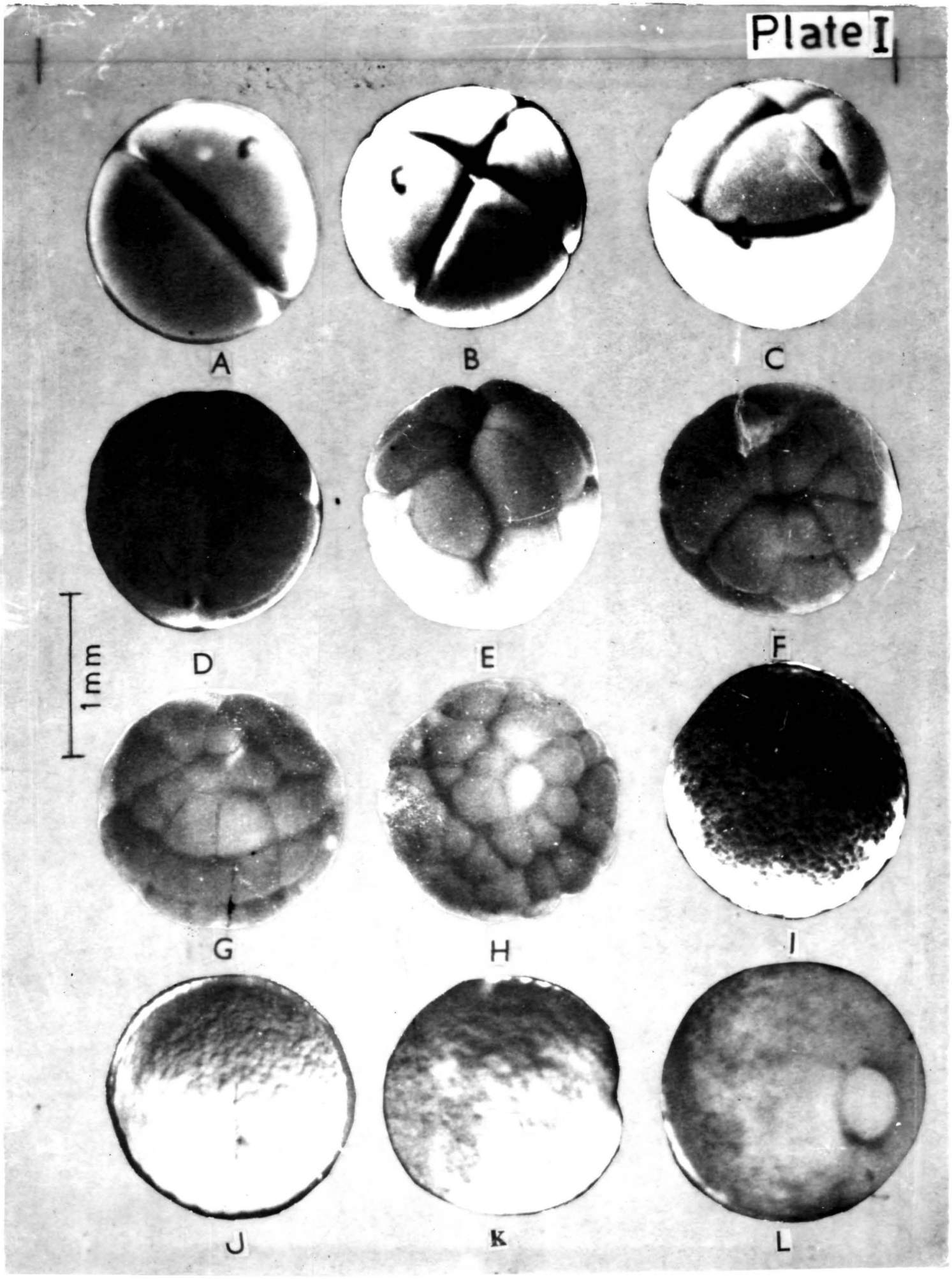


Plate II M, N, O, P and Q - Disappearing yolk plug (Stage 13).

Plate II R - Neural plate (Stage 14).

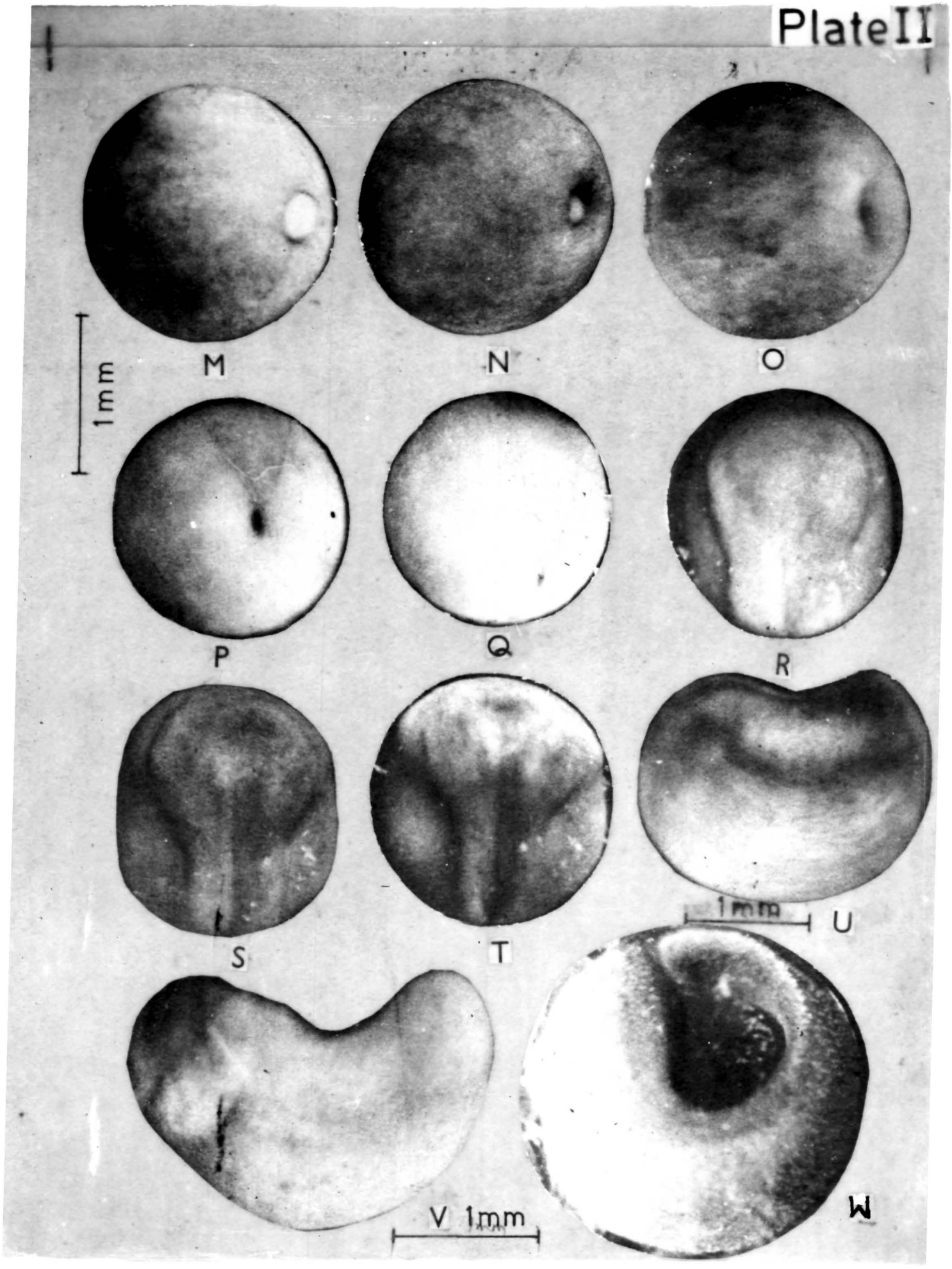
Plate II S and T - Neural fold (Stage 15).

Plate II U - Neural groove (Stage 16).

Plate II V - Tail bud (Stage 17).

Plate II W - Late tail bud (Stage 20).

Plate II



caudad, below the anterior margin of the eye ball. Tail assumes a darker and less transparent appearance than in previous Stages. The dorsal and ventral fins shrunken and the tail length considerably reduced, although still longer than the extended hind limbs. Head-Trunk length 7.0 mm. and tail length 11.00 mm. (Fig. 60 A and B).

STAGE 30: Age 722 - 770 Hour; Length 16.00 - 11.50 mm.

Tail shortened. Angle of the mouth reached the posterior region of the eye ball. Opercular patch dark and reduced. Head-Trunk length about 7.00 mm. and tail length about 4.5 mm. (Fig. 61 A and B).

STAGE 31: Age 770 - 790 Hour; Length 11.50 - 7.50 mm.

Tail only a small triangle, no longer visible from vertral side. The triangular stub of tail is darker in colour than the rest of the body. Tympanic cartilage ring present. Head-Trunk length about 7.0 mm. and tail stub about 0.50 mm. (Fig. 62 A and B).

STAGE 32: Age 790 - 900 Hour; Length 10.00 mm.

Fully metamorphosed young frog with all anuran characters. (Fig. 63 A and B).

DISCUSSION

DEVELOPMENTAL TIME

Rana limnocharis Wiegmann available at Shillong is a seasonal frog breeding mostly from the middle of April to

August. This is the best period for its induced breeding and study of its development. During this period at room temperature (14°C-22°C) its development is completed within 33 to 36 days. Hatching occurs on the 4th/5th and sometimes on 6th day. Operculum is formed on 10 to 12 days, hind limbs develop from 12 to 19 days, forelimbs develop between 19 to 30 days. The longest size of the tail is noted by about 19th day and then it is resorbed, disappearing completely by 33 to 36 days. The time of development of different stages is different from the timing of developmental stages of other frog species Rana tigrina described from this country (Agarwal and Niazi, 1977). Rana tigrina embryo has been described to hatch by about 17½ hours, whereas Rana limnocharis embryos hatches mostly on the 5th and sometimes on the 6th day at Shillong. The hatching time of Rana limnocharis is very much similar to Rana pipiens (Shumway, 1940) in which hatching occurs after about 6 days at 18°c. The metamorphosis of Rana pipiens is, however, prolonged to about 90 days (Taylor and Kollros, 1946) which is a much longer time than that of Rana limnocharis observed in the present investigation. The hatching time of Rana limnocharis investigated at Singapore at 27°C-29°C was was found by Hock and Wen (1970) to be about 24 hours. They reported 7°C and 29°C as the lower and upper limiting temperatures for Rana limnocharis which are comparable to the limiting temperatures (5°C and 28°C) investigated for

Rana limnocharis for this study at Shillong (Chapter VI). Near the upper limiting temperature the embryonic development of Rana limnocharis was found to be very fast in the present investigation also. The difference between the atmospheric temperatures at the two places may account for the difference in the hatching time of Rana limnocharis observed in the two investigations. The longer hatching time of Rana limnocharis at the subtropical climate of Shillong makes it more suitable material for the study of the embryonic stages in this region. The higher temperature (26°C-28°C) at which Agarwal and Niazi (1977) studied the development of Rana tigrina may account for its faster developmental rate also during embryonic stages and slower developmental rate at post embryonic stages.

STAGING OF EMBRYOS

The staging of embryos and larvae is normally based on a new developmental event. Although the criteria followed by different authors is similar, yet there is lot of variation in the numbering of stages of different anurans. The tables of Shumway (1940) for embryonic development and Taylor and Kollros (1946) for post embryonic development of Rana pipiens have been mostly used for reference by many investigators and laboratory manuals (Hamburger, 1960; Rugh, 1962). Some variation is always noted in the developmental patterns of body parts in different species and many

a time it becomes difficult to follow any criteria very rigidly. Gosner's (1960) table describing a criteria for staging is also sometimes not applicable in different species. There are very few tables describing the complete development of anurans. In this connection normal tables of Bufo vulgaria (Alder, 1901), Rana fusca (Kopsch, 1952), Rana dalmatina (Cambar and Marrot, 1954), Bufo melanostictus (Khan, 1965), Xenopus laevis (Nieuwkoop and Faber, 1967), Rana tigrina (Agarwal and Niazi, 1977) can be mentioned. Of all these tables the normal table of Xenopus laevis described by Nieuwkoop and Faber is most exhaustive. They have adopted external as well as internal morphological changes as the criteria for staging specially because Xenopus laevis has been found to be one of the most suitable material for developmental studies as well as experimental and transplantation work in many laboratories of Western countries. In the present investigation the criteria adopted by Nieuwkoop and Faber for external morphological changes has been followed. The description of internal changes was not at present considered necessary. The development of Rana limnocharis in the present investigation has been divided into 20 embryonic and 12 post embryonic stages. The present investigation on Rana limnocharis reveals that (1) it responds well to prescribed dosage of homoplastic pituitaries preserved in absolute ethyl alcohol for inducing ovulation in females during breeding seasons provided the

specimens used are not spent; (2) its life cycle is completed in approximately 5 weeks; (3) its embryonic period is comparatively longer and post embryonic period is shorter; (4) it is easy to work with this species due to its handy size and (5) the 32 developmental stages described above can be conveniently demonstrated. It is therefore, hoped that this species can be a convenient classroom material for developmental studies in Indian conditions.

SUMMARY

The present chapter embodies the description of normal table of development of Rana limnocharis Wiegmann by rearing under laboratory condition through induced breeding. The staging of the embryos has been based on major changes in the external morphological features. The entire development from fertilized egg to metamorphosed froglet has been divided into 32 distinct stages, fertilization :- stages 1 and 2; cleavage :- stages 3 to 8; gastrulation :- stages 9 to 12; neurulation :- stages 13 to 15; organogenesis :- stages 16 to 20; and post-embryonic stages 21 to 32. Rana limnocharis is a seasonal frog breeding from the middle of April to August in Shillong. At the room temperature fluctuating between 14°C (night) and 22°C (day), during the early breeding period, the development was completed in 33 to 36 days. Hatching occured on the 4th/5th day and sometimes on the 6th day. The larva developed operculum between 10th and 12th day and subsequently underwent metamorphosis. Hindlimbs developed during the period, 12th to 19th day. Forelimbs were formed between the 19th and 30th day. The tail reached maximum size on about 19th day after which it gradually resorbed, disappearing completely between 33rd and 36th day.

REFERENCES

Alder, W. 1901. Die Entwicklung der äusseren Körperform und des Mesoderms be Bufo vulgaris. Internatl. Monatschr. Anat. Physiol. 18: 19-51.

Agarwal, S.K. and I.A. Niazi. 1977. Normal table of developmental stages of the Indian bullfrog, Rana tigrina Daud. (Ranidae, Anura, Amphibia). Proc. Nat. Acad. Sci. India. 47(B): 79-91.

Billet, F.S. and A.E. Wild. 1975. Practical studies on animal development. Chapman and Hall. London.

Bretscher, A. 1949. Die Hinterbeinenwicklung von Xenopus laevis Daud - und ihre Beeinflussung durch Colchicin. Rev. Suisse Zool. 56: 34-96.

Cambar, R. and Br. Marrot. 1954. Table chronologique du développement de la grenouille agile (Rana Dalmatina Bon). Bull. Biol. Franco - Belg. 88: 168-177.

Cambar, R. and J.D. Gipouloux. 1956. Table chronologique du développement embryonnaire et larvaire du crapaud commun : Bufo bufo L. Bull. biol. Fr Belg. 90: 198-217.

Cambar, R. and S. Martin. 1959. Table chronologique du développement embryonnaire et larvaire du crapaud accoucheur (Alytes obstetricans Laur.) Act. Soc. Linn. Bordeaux. 98: 1-20.

Del Conte, E. and J.L. Sirlin. 1952. Pattern series of the first embryonary stages in Bufo arenarum. Anat. Rec. 112: 125-135.

Eakin, R.M. 1946. Determination and regulation of polarity in the retina of Hyla regilla. Univ. California Publ. Zool. 51: pp 245.

Gallien, L. and Ch. Houillon. 1951. Table chronologique du développement chez Discoglossus pictus. Bull. Biol. Franco - Belg. 85: 373-375.

Gasche, P. 1944. Beginn und Verlauf der Metamorphose bei Xenopus laevis Daud. Festlegung von Umwandlungsstadien. Helvetica Physiol. et Pharmacol. Acta. 2: 607-626.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica. 16: 183-190.

Hamburger, V. 1960. A manual of experimental embryology. The University of Chicago Press.

Hing, L.K. 1959. The breeding habits and development of Rana chalconata (Schleg) (Amphibia). Treubia 25: pp 89.

Hock, L.S. and C.T. Wen. 1970. Artificial breeding and early development ofthe tadpoles of Rana limnocharis Boie. J. Singapore National Academy of Science. 2(2): 59-67.

Jorquera, B. and L. Izquierdo. 1964. Tabla de desarrollo normal de Calyptocephalella gayi (Rana chilena). Biologia. (Santiago). 36: 43-53.

Khan, M.S. 1965. A normal table of Bufo melanostictus Schneider. Biologia (Lahore). 11: 1-39.

Kopsch, F. 1952. Die Entwicklung des braunen Grasfrosches Rana fusca Roesel (dargestellt in der Art der Normentafeln zur Entwicklungs-geschichte der Wirbelthiere). Stuttgart.

Limbaugh, B.A. and E.P. Volpe. 1957. Early development of the Gulf coast toad, Bufo valliceps Wiegmann. Am. Mus. Novit. 1842: 1-32.

Michniewska - Predygier, Z. and A. Pigon. 1957. Early developmental stages of Rana temporaria L., Rana terristris., Rana esculenta L., and Bufo bufo (L). Studia Soc. Sci. Torun. 3: 147-157.

New, D.A.T. 1966. The culture of Vertebrate Embryos. Logos, London.

Nieuwkoop, P.D. and J. Faber. 1967. Normal table of Xenopus laevis (Daudin). North Holland Publ. Co., Amsterdam.

Peter, K. 1931. The development of the external features of Xenopus laevis, based on material collected by the late E.J. Bales. Jour. Linn. Soc. Zool. 37: 515-523.

Pollister, A.W. and J.A. Moore. 1937. Tables for the normal development of Rana sylvatica. Anat. Rec. 68(4): 489-496.

Ramaswami, L.S. and A.B. Lakshman. 1959. The skipper-frog as a suitable embryological animal and an account of the action of mammalian hormones on spawning the same. Proc. nat. Inst. Sci. India. 25B: 68-79.

Rugh, R. 1962. Experimental Embryology. Burgess Publ. Co. Minneapolis, Minnesota.

Sedra, S.N. and M.I. Michael. 1961. Normal table of the egyptian toad, Bufo regularis Reuss, with an addendum on the standardization of the stages considered in previous publications. Ceskoslovenská Morf. 9: 333-351.

Siboulet, R. 1970. Table chronologique du développement embryonnaire et larvaire du crapaud dé Mauretanie Bufo mauretanicus Schlegel, 1841, á différéntes températures. Vie Milieu. Ser. C. 21: 179-198.

Shumway, W. 1940. Stages in the normal development of Rana pipiens. I. External form. Anat. Rec. 78(2): 139-147.

Tahara, Y. 1959. Table of the normal developmental stages of the frog, Rana japonica. I. Early development. (Stages 1-25). Jap. J. exp. Morph. 13: 49-60.

Taylor, A.C. and J.J. Kollros. 1946. Stages in the normal development of Rana pipiens larvae. Anat. Rec. 94(1): 7-23.

*Not consulted in original.

Chapter 3

Food and Feeding Habits

INTRODUCTION

In recent years the need to evolve convenient and economical technique for frog culture has been increasingly felt by workers all over the world. One important prerequisite in this context is to get acquainted with the food and feeding habits of the frog species to be cultured in a particular locality. Tinsley (1973) and Clarke (1974) stated that pattern of exploitation of prey is an important characteristic of different groups of anura. Hedeen (1972) and Brown (1974) analysed that, food consumed by anurans reflect the abundance and availability of prey in its habitat. Blackith and Speight (1974) investigated that frog's gape and movement pattern are responsible for determining the diet in a particular habitat.

In comparison to the number of contributions on food and feeding habits of anuran species found in the temperate region, there are a few contributions from the tropical region (Durant and Dole, 1974) and very few from the Indo-Pak subcontinent (Khan, 1973).

The subtropical climate of Shillong and the neighbouring hills provide a specialised cold and humid biotope in the North-Eastern region of India. As stated earlier Rana limnocharis Wiegmann is a common species of frog in this region and is eaten by some tribes of the North-Eastern Hills States. It is therefore, a suitable species

for culturing, both from academic as well as economic point of view. Among earlier works there are two contributions available on the food and feeding habits of this species. Liu and Chen (1932) investigated the percentage composition of injurious and beneficial animals in the food contents of Rana limnocharis available in the vicinity of Kashing (China) with special reference to insects in relation to paddy cultivation. Berry (1965) investigated the gut contents of Rana limnocharis from Singapore. He reported a high percentage of vegetable matter in their stomach contents, in addition to animals ranging from small to large sizes depending upon the mouth gape and food availability in the habitat. The present study is based on a 12-month analysis of the volume and percentage composition of food taken by the adult and juveniles of Rana limnocharis during the prebreeding, breeding, postbreeding and hibernation periods.

REVIEW OF LITERATURE

Gut content analysis reveals the food and feeding habit of animals. Among anurans, the first detailed analysis of stomach contents was made by Liu and Chen (1932) on the two species of frogs, Rana limnocharis and Rana nigromaculata available in the vicinity of Kashing (China), situated just above the sea level, with special reference to insects.

They studied the percentage composition of beneficial and injurious animals in the food contents and relationship of these animals with the paddy cultivation. Smith (1953) studied the feeding habits of Rana ridibunda ridibunda and showed that, in their choice of food they were extremely catholic and would not devour very fine creatures. Tyler (1958) worked in France on the diet, feeding habits and preying behaviour of the edible frog Rana esculenta. In 1961, Khonyakina working in the vicinity of Makhachkala (Russia) on the feeding habits of Rana ridibunda and Bufo viridis reported that, they fed mainly on arthropods specially agricultural pests proving their utility to agriculture, although they were non-selective feeders. He also noted that Rana ridibunda causes some damage to pisciculture in shallow water reservoirs. Berry (1965) worked on the diet of six anuran species Rana limnocharis, Rhacophorus leucomystax, Microhyla butleri, Microhyla hymensii, Kaloula pulcra and Leptobrachium nigrops available in Singapore and reported various degrees of selectivity for particular type of prey. He reported that availability of prey in the habitat, the size of prey and of predators especially in relation to the mouth-gape determined in the diet. In 1966, he published his findings on the food and feeding habits of the torrent frog, Amolops larutensis in Kaulalumpur, and showed that these frogs feed on a wide range of terrestrial and aquatic

animals. They showed preference for prey animals within a range of 1.00 - 6.5 mm. He did not find any clear seasonal variation in the diet and suggested that monthly fluctuations in the amount and type of prey ingested depended largely on the activity of the prey. Jenssen and Klimstra in the same year noted that food of Rana clamitans is affected by habitat. Jenssen (1967) reported that Rana clamitans larvae were indiscriminate feeder of food available in their habitat. Guyetant, during the same year, reported that there was variation in the diet of young and adult Rana temporaria, Rana esculenta and Bufo bufo concluding that feeding habits probably continue to alter. Opatrny (1968) reported that the terrestrial insects and animals living on water surface predominated as the food items of Rana ridibunda and Rana esculenta found in central Bohemia, South Slovakia and South Eastern Romania depending on the biotope. He also recorded cannibalism. Chlodny (1969) worked on the food requirement of Rana arvalis in Poland and showed that efficiency of assimilation is constant for a given diet being 84.6% when fed on earthworms and 73.3% when fed on insects under laboratory conditions. Franz (1970) found that the food of larval tailed frog, Ascaphus truei consisted mainly of algae. Pengiliey (1970) worked on the food and some Australian anurans of Southern Highlands of New South Wales. He examined the frequency and percentage of food items taken out from stomachs of juvenile,

subadult and adult of three species of genus Pseudophryne and showed that their diet consisted mainly of ants and termites. He found that the size of prey items was associated with the size of the predator and seasonal variations, especially changes in temperature, influenced the feeding activity of the adults. In 1972, Hedeen reported that Rana septentrionalis Baird, the mink frog, available in Minnesota fed on different diets during different stages of the development depending on seasonal food availability and changes in the habitat. Stewart and Sandison (1972) made a comparative study of food habits of sympatric mink frog Rana septentrionalis, bull frog Rana catesbiana, green frog Rana clamitans and found that although major food groups for both mink frog and green frog were similar, there were distinct differences in total diet. Bruggers (1973), as a result of his work on food and feeding habits of bull frog Rana catesbiana of North-Western Ohio, found that they ate everything that they could swallow. Houston (1973) studied the food of common frog, Rana temporaria, on high moorland of Northern England. He showed that there was a temporal variation of food during different periods of growth of the frog. He suggests that there is a qualitative correlation between the size of the prey and predator. He also found out that the animal is unspecialised and opportunistic feeder in its habitat. Kalusche (1973) worked on the diet of Rana esculenta and noted that it snapped at and ate

tadpole in shallow water ditches in terrarium. During the same year, Khan investigated that Rana tigrina was an indiscriminate feeder and its mouth-gape determined maximum size of the prey. Tinsley (1973) while studying the ecology and systematics of Xenopus kigesiensis from Western Uganda took stomach contents also as a criteria for describing the species. Blackith and Speight (1974) reported Rana temporaria as an effective predator of ground living predatory and parasitic insects. Durant and Dole, during the same year reported that food of Atelopus exyrhynchus consisted mainly of coleopterans (40%), while hymenoptera (mostly ants), larval diptera and acarina comprise (45%) and other food items were (15%). They further described that amplexing frogs had much less food material in their stomach. Cristea (1974) investigated the food of green frog, Rana ridibunda and Rana esculenta. Living in Danube's delta and flooded land. He showed that larval stage was mainly dependent on filamentous algae and vegetal detritus. Up to juvenile stage these frogs lived on submerged green plants and algae, copepoda, cladocera, rotifera and chironomus larvae. Their adults lived on animal diet. Elliot and Karunakaran (1974) investigated the diet of Rana cancrivora in fresh and brackish water environments of Singapore and showed that the gut content included all small animal species found in the respective environments. The diet of frogs near brackish water consisted of predominantly

crustaceans, while the diet of those collected near fresh water comprised mainly of insects. The choice of the prey limited only by its size and the mouth-gape of the predator. Tucker and Sullivan (1975) reported unsuccessful attempts of Rana catesbiana to eat Bufo as the latter appeared unpalatable. Bailey (1976) recorded that the main food item of the toad Bufo marinus comprised 80% snails and ants. Labanick (1976) while working upon the prey availability, consumption and prey selection in the cricket frog Acris crepitans reported that mean prey size increased linearly and mean number of items per stomach decreased with the increasing size of the frog. He analysed by rank analysis that prey selection was not as important as the prey availability.

MATERIALS AND METHODS

The study of the food and feeding habits of Rana limnocharis was based on the gut content analysis of 280 specimens collected during 1976-77 from the Pologround at regular intervals throughout the year. During hibernation the frogs were collected from burrows and crevices below the stones. After collection, the frogs were immediately killed by chloroform and preserved in 70% alcohol to check any further digestion of the food contents. Before preservation a small incision was made in the abdomen of

the specimens. The specimens collected were divided into two groups: adults (males SVL above 3.10 cm and females SVL above 4.15 cm) and juveniles having SVL less than that of adults. They were weighed and dissected. Size of various parts of the alimentary canal was recorded. The food materials were taken out separately from the stomach, intestine and rectum and transferred to a graduated cylinder having 5.00 ml water. The volume of the food content was determined by the amount of water displaced. The food items were next poured in a watch glass and examined under dissecting binocular microscope. At first a detailed analysis of the food items was made during the breeding season and then percentage composition of every item was calculated every month. The observations were made to check complete as well as fragmented food items. In all 20 adults and 20 juveniles were examined during breeding period. For investigating the annual changes of the food intake 20 adults were examined every month. The animals taken as food items were identified up to orders, most to families and some to species. During breeding season analysis of the food items present in the intestine and rectum was also made with a view to find out as to which food items were completely digested and which partly digested.

OBSERVATIONS

Observations were made on body weight, snout vent length, gut length, food volume and frequency and percentage composition of food contents.

A. ANALYSIS DURING THE BREEDING PERIOD.

1. Body weight, body length, gut length and food volume :

(a) ADULTS (Table I)

The 20 adult specimens studied, weighed from 3.05 gm to 13.90 gm. The males being 3.05 gm to 4.85 gm, whereas females from 7.10 gm to 13.90 gm. The corresponding S-V length ranged from 3.10 cm to 5.35 cm: males being 3.10 cm to 4.00 cm and females being 4.15 cm to 5.35 cm. The body breadth ranged from 1.60 cm to 2.35 cm; being 1.35 cm to 2.20 cm in males and 1.30 cm to 2.35 cm in females. The head length ranged from 0.50 cm to 1.30 cm; being 0.50 cm to 1.60 cm in males and 0.65 cm to 1.30 cm in females. The mouth-gape ranged from 0.95 cm to 1.75 cm; being 0.95 cm to 1.60 cm in males and 0.90 cm to 1.75 cm in females.

The gut length ranged from 9.20 cm to 17.70 cm; being 9.80 cm to 14.70 cm in the males and 9.20 cm to 17.70 cm in the females. The length of the stomach ranged from 1.30 cm to 2.55 cm; being 1.30 cm to 2.55 cm in the males and 1.65 cm to 2.50 cm in the females. The intestine length varied from 5.00 cm to 13.90 cm; being 6.95 cm to 10.05 cm in the males and 5.00 cm to 13.90 cm in the females. The rectum

TABLE I

Rana limnocharis Wiegmann: Dimensions and volume of gut content of the adults during the breeding period

Measurements	Male (9) Average (range)	Female (11) Average (range)	Total adult (20) Average (range)
Body weight (gm)	4.68 (3.05- 4.85)	9.60 (7.00-13.90)	7.39 (3.05-13.90)
Body length SVL (cm)	3.60 (3.10- 4.00)	4.82 (4.15- 5.35)	4.50 (3.10- 5.35)
Body breadth (cm)	1.80 (1.35- 2.20)	2.02 (1.30- 2.35)	1.92 (1.60- 2.35)
Head length (cm)	0.96 (0.50- 1.30)	1.06 (0.65- 1.30)	1.02 (0.50- 1.30)
Mouth-gape (cm)	1.25 (0.95- 1.60)	1.40 (0.90- 1.75)	1.34 (0.95- 1.75)
Total length of gut (cm)	11.46 (9.80-14.70)	13.74 (9.20-17.70)	12.71 (9.20-17.70)
Stomach length (cm)	1.72 (1.30- 2.55)	2.05 (1.65- 2.50)	1.90 (1.30- 2.55)
Intestine length (cm)	8.43 (6.95-10.05)	10.14 (5.00-13.90)	9.37 (5.00-13.90)
Rectum length (cm)	1.31 (0.75- 2.10)	1.65 (0.75- 2.70)	1.50 (0.75- 2.70)
Total volume of food contents in gut (ml)	0.41 (0.20- 0.60)	0.63 (0.25- 0.80)	0.53 (0.20- 0.80)
Total volume of food contents in stomach (ml)	0.21 (0.05- 0.04)	0.32 (0.10- 0.70)	0.27 (0.05- 0.70)
Total volume of food contents in intestine (ml)	0.10 (0.05- 0.20)	0.14 (0.05- 0.30)	0.12 (0.05- 0.30)
Total volume of food contents in Rectum (ml)	0.10 (0.05- 0.30)	0.18 (0.05- 0.40)	0.14 (0.05- 0.40)

length ranged from 0.75 cm to 2.70 cm, the length of rectum in the males being 0.75 cm to 2.10 cm and in the females 0.75 cm to 2.70 cm.

The total food taken by different individuals ranged from 0.20 ml to 0.80 ml by volume, being 0.20 ml to 0.60 ml in the males and 0.25 ml to 0.80 ml in the females. The volume of food contents varied in different parts of the gut in different specimens examined. In stomach it ranged from 0.05 ml to 0.70 ml; in intestine it ranged from 0.05 ml to 0.30 ml and in rectum 0.05 ml to 0.40 ml. In the males the volume of the stomach contents measured from 0.05 ml to 0.40 ml; in the intestine 0.05 ml to 0.20 ml and in the rectum from 0.05 ml to 0.30 ml. In the females the volume of the stomach contents measured from 0.10 ml to 0.70 ml; in the intestine contents from 0.05 ml to 0.30 ml and in the rectum from 0.05 ml to 0.40 ml.

(b) JUVENILES (Table II)

The weight of the 20 juveniles specimens examined ranged from 1.70 gm to 4.90 gm; the males being 1.70 gm to 3.10 gm and the females 2.60 gm to 4.90 gm. The corresponding body length (SVL) of these specimens being 2.60 cm to 3.90 cm; males being 2.60 cm to 3.05 cm and females 3.00 cm to 3.90 cm. The body breadth ranged from 0.85 cm to 2.00 cm; being 0.85 cm to 1.55 cm in males and 1.00 cm to 2.00 cm in females. The head length ranged from 0.60 cm to 1.00 cm:

TABLE II

Rana limnocharis Wiegmann: Dimensions and volume of gut content of the juveniles during the breeding period

Measurements	Male (11) Average (range)	Female (9) Average (range)	Total young (20) Average (range)
Body weight (gm)	2.95 (1.70-3.10)	3.34 (2.60- 4.90)	3.12 (1.70- 4.90)
Body length SVL (cm)	3.01 (2.60-3.05)	3.27 (3.00- 3.90)	3.16 (2.60- 2.00)
Body breadth (cm)	1.16 (0.85-1.55)	1.47 (1.00- 2.00)	1.30 (0.85- 2.00)
Head length (cm)	0.77 (0.60-1.00)	0.81 (0.60- 1.00)	0.79 (0.60- 1.00)
Mouth-gape (cm)	1.14 (0.95-1.30)	1.14 (1.00- 1.50)	1.12 (0.95- 1.50)
Total length of gut (cm)	8.45 (7.40-9.35)	9.95 (7.50-13.10)	9.12 (7.40-13.10)
Stomach length (cm)	1.34 (1.10-1.40)	1.51 (1.30- 1.90)	1.42 (1.10- 1.90)
Intestine length (cm)	5.99 (4.90-6.75)	7.16 (4.80-10.50)	6.52 (4.80-10.50)
Rectum length (cm)	1.09 (0.85-1.35)	1.27 (0.95- 1.60)	1.17 (0.85- 1.60)
Total volume of food contents in gut (ml)	0.32 (0.15-0.60)	0.35 (0.30- 0.40)	0.33 (0.15- 0.60)
Total volume of food contents in stomach (ml)	0.16 (0.05-0.30)	0.16 (0.10- 0.20)	0.16 (0.05- 0.30)
Total volume of food contents in intestine (ml)	0.08 (0.05-0.10)	0.09 (0.05- 0.10)	0.08 (0.05- 0.10)
Total volume of food contents in rectum (ml)	0.09 (0.05-0.20)	0.11 (0.05- 0.20)	0.10 (0.05- 0.20)

being 0.60 cm to 1.00 cm in males and 0,60 cm to 1.00 cm in females. The mouth-gape ranged from 0.95 cm to 1.50 cm; being 0.95 cm to 1.30 cm in males and 1.00 cm to 1.50 cm in females.

The gut length in these specimens varied from 7.40 cm to 13.10 cm: being 7.40 cm to 9.35 cm in the males and 7.50 cm to 13.10 cm in the females. The size of different regions of the gut also varied in different specimens. The stomach length of the juvenile varied from 1.10 cm to 1.90 cm, in which the males measured 1.10 cm to 1.40 cm and in the females 1.30 cm to 1.90 cm. The intestine length measured from 4.80 cm to 10.50 cm, which in males was 4.90 cm to 6.75 cm and in females 4.80 cm to 10.50 cm. The length of the rectum varied from 0.85 cm to 1.60 cm; 0.85 cm to 1.35 cm in the males and 0.95 cm to 1.60 cm in the females.

The total food taken by different individuals ranged from 0.15 ml to 0.60 ml by volume, being 0.15 ml to 0.60 ml in the males and 0.30 ml to 0.40 ml in the females. The volume of food contents varied in the different parts of the gut. In the stomach, the volume of the food contents varied from 0.05 ml to 0.30 ml; in the intestine 0.05 ml to 0.10 ml and in the rectum 0.05 ml to 0.20 ml. In males, in the stomach it measured to be 0.05 ml to 0.30,ml; in the intestine 0.05 ml to 0.10 ml and in the rectum 0.05 ml to 0.20 ml. In the females the volume of the stomach contents

Fig. 1 - Percentage composition of complete food items found in the gut of adult and juvenile Rana limnocharis Wiegmann.

Abbreviations:

HYM - Hymenoptera	OLG - Oligochaeta
COL - Coleoptera	ONS - Oniscoidea
ARA - Arachnida	PEN - Penaedea
GRY - Gryllotalpidae	DIC - Dictyoptera
TET - Tettigonidae	DIP - Diptera
LEP - Lepidoptera	MOL - Mollusca
LAR - Insect larvae	HEM - Hemiptera

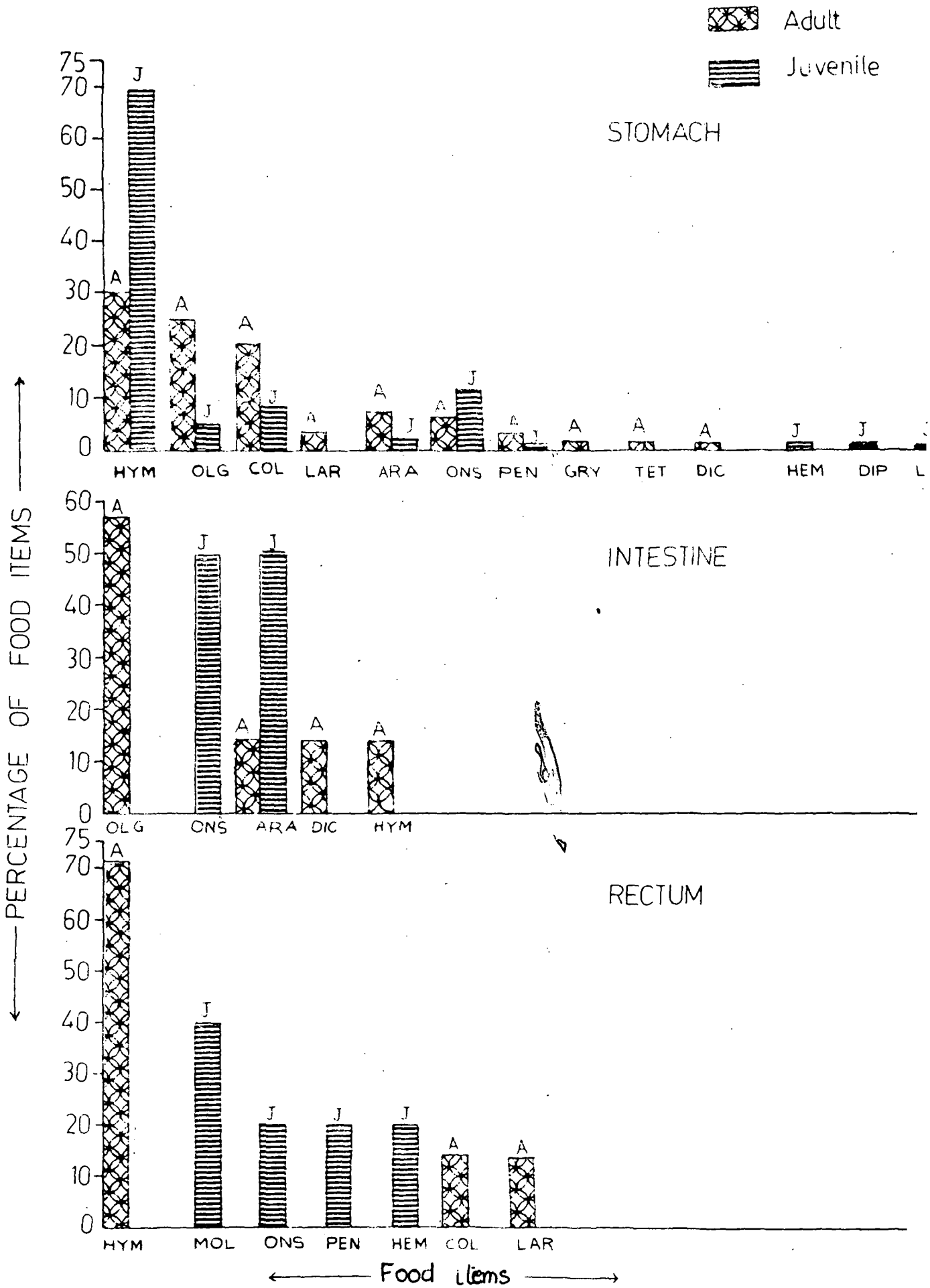

Adult
Juvenile
STOMACH
INTESTINE
RECTUM
PERCENTAGE OF FOOD ITEMS
Food Items
HYM
OLG
COL
LAR
ARA
ONS
PEN
GRY
TET
DIC
HEM
DIP
MOL

Fig.1

Fig. 2 - Percentage composition of fragmentary food items found in the gut of adult and juvenile Rana limnocharis, Wiegmann.

Abbreviations:

LAR - Insect larvae	COL - Coleoptera
HYM - Hymenoptera	ONS - Oniscoidea
ARA - Arachnida	OLG - Oligochaeta
DIC - Dictyoptera	PEN - Penaedea
HEM - Hemiptera	STO - Stones
LEP - Lepidoptera	MOL - Mollusca
RSH - Rotifer shell	DIP - Diptera
GRY - Gryllotalpidae	GSS - Grass, Straw, Stones

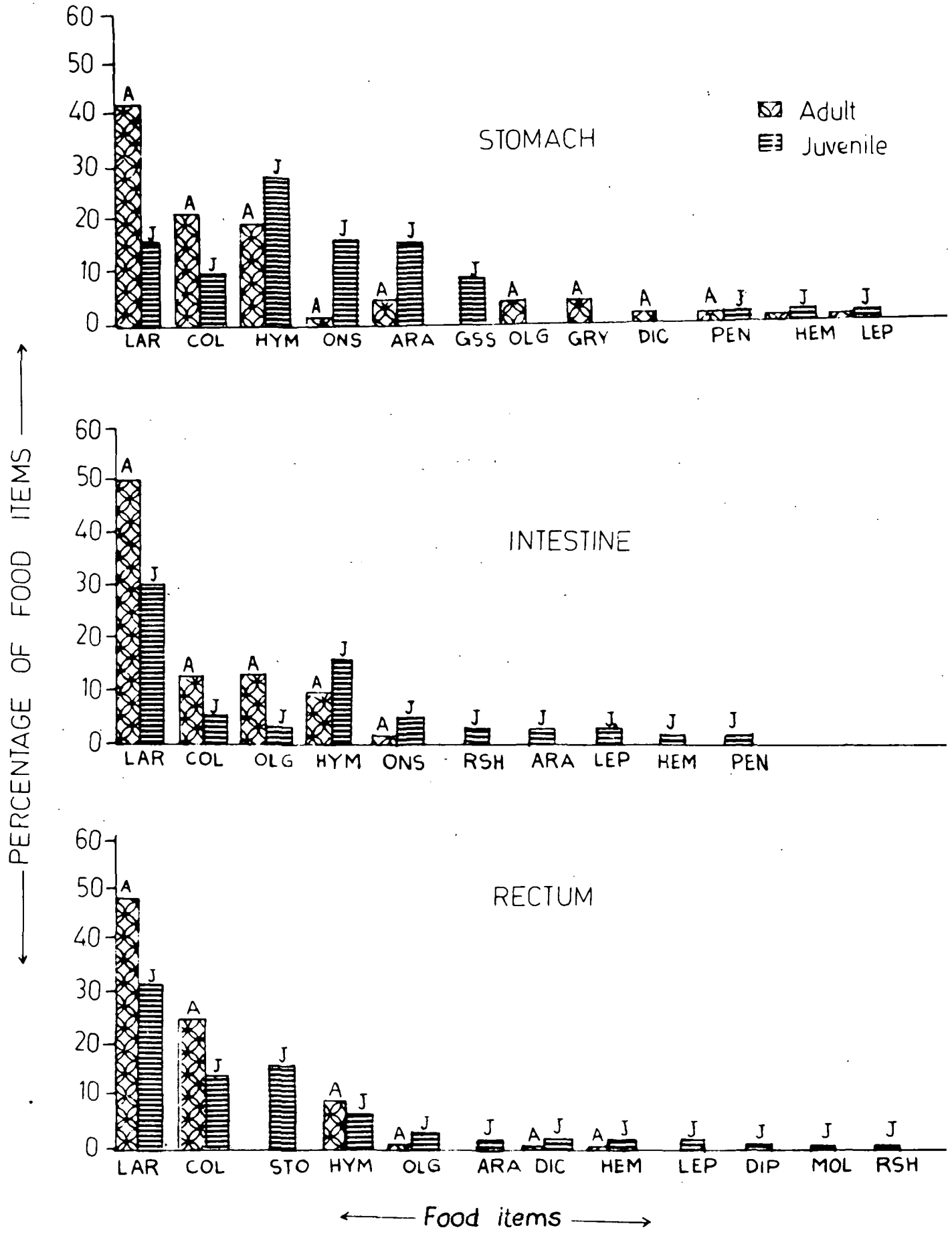

STOMACH
Adult
Juvenile
60
50
40
30
20
10
0
A
J
LAR COL HYM ONS ARA GSS OLG GRY DIC PEN HEM LEP
INTESTINE
LAR COL OLG HYM ONS RSH ARA LEP HEM PEN
RECTUM
LAR COL STO HYM OLG ARA DIC HEM LEP DIP MOL RSH
PERCENTAGE OF FOOD ITEMS
Food items

Fig. 2

varied from 0.10 ml to 0.20 ml, in the intestine from 0.05 ml to 0.10 ml and in the rectum from 0.05 ml to 0.20 ml.

2. Analysis of the food items :

Following food items were recorded from stomach, intestine and rectum during breeding season. (Tables III, IV, V and VI; Figs. 1 and 2).

(a) STOMACH

The analysis of the food items of stomach revealed the nature of items taken as food. The content of the stomach comprised complete specimens of fragments of the oligochaetes, arachnids, crustaceans, insects, vegetable matter and small stones, in the adults as well as juveniles as described below. Identification up to species level could not be done in certain cases.

1. Annelida
   Oligochaeta
   Earthworms

The Oligochaeta were represented by earthworms in the food contents. The earthworms were identified as vermiform, metamerically segmented animals, having clitellum. In adult frogs, the complete specimens comprised 25.28% of the food content and the fragments 4% of the total fragmentary contents of the stomach.

In the juvenile frogs, the earthworms formed 5.39% of the food content. Fragmentary parts were not found in the juveniles.

TABLE III

Rana limnocharis Wiegmann: Complete food items obtained from the gut of the adults.

Food Items	STOMACH		INTESTINE		RECTUM	
	Percentage Composition	Frequency of occurence	Percentage composition	Frequency of occurence	Percentage composition	Frequency of occurence
Annelida						
Oligochaeta	25.28	5	57.14	2		
Arthropoda						
Arachnida Arachnida	8.04	4	14.28	1		
Crustacea						
Isopoda Oniscoidea	6.89	3				
Decapoda Penaedea	3.44	2				
Insecta						
Orthoptera						
Gryllotalpidae	1.49	1				
Tettigonidae	1.49	1				
Dictyoptera Blattaria	1.49	1	14.28	1		
Hymenoptera	30.22	12	14.28	1	71	2
Coleoptera	20.68	9			14	1
Insect larvae	2.29	2			14	1

TABLE IV

Rana limnocharis Wiegmann: Fragmented food items obtained from the gut of the adults

Food Items	STOMACH		INTESTINE		RECTUM	
	Percentage composition	Frequency of occurence	Percentage composition	Frequency of occurence	Percentage composition	Frequency of occurence
Annelida						
Oligochaeta	4	4	13	7		
Arthropoda						
Arachnida Araneae	5	4				
Crustacea						
Isopoda Oniscoidea	1	1	2	1	1	1
Decapoda Penaedea	2	2				
Insecta						
Gryllotalpidae	4	4				
Dictyoptera Blattaria	2	1			1	1
Hemiptera	1	1			1	1
Hymenoptera	19	8	10	4	10	4
Coleoptera	21	1	13	4	25	7
Insect larvae	42	19	50	12	48	14
Nematode			12	6	13	10

TABLE V

Rana limnocharis Wiegmann: Complete food items obtained from the gut of the juveniles.

Food Items	STOMACH		INTESTINE		RECTUM	
	Percentage composition	Frequency of occurence	Percentage composition	Frequency of occurence	Percentage composition	Frequency of occurence
Annelida						
Oligochaeta	5.39	5				
Arthropoda						
Arachnida Araneae	1.47	15	50	1		
Crustacea						
Isopoda Oniscoidea	11.76	8	50	1	20	1
Decapoda Penaedea	0.98	2			20	1
Insecta						
Hemiptera	1.47	3			20	1
Lepidoptera	0.49	3				
Diptera	0.98	1				
Hymenoptera	69.60	16				
Coleoptera	7.35	10			40	2
Mollusca	0.49	1				

TABLE VI

Rana limnocharis Wiegmann: Dimensions and volume of gut contents of the adults during the breeding period.

Food Items	STOMACH		INTESTINE		RECTUM	
	Percentage composition	Frequency of occurence	Percentage composition	Frequency of occurence	Percentage composition	Frequency of occurence
Rotifer shell			3.38	2	1.29	1
Annelida						
Oligochaeta			3.38	2	3.38	3
Arthropoda						
Arachnida Araneae	15.06	8	3.38	2	2.69	2
Crustacea						
Isopoda Oniscoidea	16.43	10	5.08	3	1.29	1
Decapoda Penaedea	1.36	1	1.69	1	2.59	1
Insecta						
Hemiptera	2.73	2	1.69	1	2.59	2
Lepidoptera	2.73	1	3.38	2	2.59	2
Diptera					1.29	1
Hymenoptera	28.75	13	16.94	8	7.79	5
Coleoptera	9.50	3	5.08	1	14.28	8
Insect larvae	15.06	10	30.50	13	31.16	15
Mollusca					1.29	1
Grass, Straw and Stones (taken as food)	8.20	6			16.88	13
Nematode			8.47	5	7.79	6

2. Arthropoda
   Arachnida
   Araneae
   Spiders

The Araneae includes spiders. These spiders formed a part of the diet of the frog. The spiders were identified by the presence of cephalothorax and abdomen connected to each other by a slender pedicel and six pairs of cephalothoracic appendages of which the first pair called the chelicerae were prominent. The spiders comprised 8.04% of the food content of the adult frogs. Numerous fragments such as cephalothorax, abdomen, eyes, chelicerae and different parts of the appendages comprised 5% of the total fragmented components obtained from the stomach.

In the juveniles, the whole animal represented 1.47% of the total stomach contents, while the fragments of the spider body showed a greater percentage of 15.06% of the total fragmentary components of the stomach.

3. Crustacea
   Isopoda
   Oniscoidea
   Oniscus

The Oniscoidea was represented in the diet of the frog by the oniscus, identified by dorsoventrally flattened body, having distinct segmentation, ventigeal antennules, absence of carapace, uniramous thoracic

appendages and last abdominal segment fused with the telson. In the adult frogs, Oniscus formed 6.89% of the total food items, where as the fragmented parts of the animal made up 1% of the total fragmented components.

The stomach content of the juveniles showed that they had a better preference for Oniscus than the adults, which formed 11.76% of the total stomach contents. The fragmented parts of this animal made up 16.43% of the total fragmentary stomach content.

4. Crustacea
   Decapoda
   Penaeidea
   Prawn

The small prawns (Macrobrachium species) were identified by a cephalothorax, with carapace overhanging the lateral sides to form a branchial chamber, usually without a rostrum and three pairs of chelate legs. They formed 3.44% of the complete food items found in the adult. The fragmentary parts of the cephalothorax, legs or the carapace comprised 2% of the total fragmentary components.

Whole specimens of the prawns were represented in the juveniles by a small percentage of 0.98% of the total components of the stomach and the fragments being 1.36% of the total fragmentary components.

5. Insecta
   Orthoptera
   Gryllotalpidae
   Gryllotalpa

Gryllotalpa were identified by the biting and chewing type of mouth parts and large prothorax, legs modified for digging. The Gryllotalpa formed 1.49% of the total food items and 4% of the fragmented parts in the stomach of the adult frogs. This groups was not found in the juvenile frogs.

6. Insecta
   Orthoptera
   Tettigonidae
   Grasshoppers

Tettigonid grasshoppers were identified with long horns, long ovipositor, segmented tarsi and long antennae. They made up 1.49% of the total stomach content in the adult frogs. They were not found in the juveniles.

7. Insecta
   Dictyoptera
   Blattaria
   Periplaneta

The common cockroaches (Periplaneta) belonging to Blattaria, dark brown in colour, having rudimentary wings formed 1.49% of the stomach contents. Numerous small pieces of different parts of the body were

found in the stomach which made up 2% of the total fragmentary components of the stomach. They were not found in the juveniles.

8. Insecta
   Hemiptera
   Bugs and Aphids

In the stomach contents, Hemiptera is mostly represented by the plants bugs, aphids and water bugs identified by piercing and sucking mouth parts and typical hemipterous wings (or apterus condition in aphids). In the adult frogs, the hemipterans were represented by fragments, forming 1% of the total fragmentary stomach contents.

In the juveniles, they were represented as complete food item, forming 1.47% of the total stomach content.

9. Insecta
   Lepidoptera
   Butterflies and moths

The butterflies and moths, were identified by coiled proboscis and caterpillars or obtect pupae either free or in cocoons. These were not found in stomach content of the adult frogs. They formed 0.49% of the total complete components of the stomach in the juveniles. Fragmented parts of the lepidopterans make 2.73% of the total fragmented components.

10. Insecta
Diptera

The representatives of the order Diptera were identified by one pair of membranous wings, one pair of halteres and sponging or sucking or piercing type of mouth parts. Typical dipteran larvae and obtect or exarate pupae were also observed. The dipterans were found only in the juvenile frogs, comprising about 0.98% of the complete food items.

11. Insecta
Hymenoptera
Ants, Bees and Wasps

The Hymenoptera were represented in the stomach contents by the ants, bees and wasps. They were identified by membranous wings, the hind pair being smaller and connected with the fore pair by hooklets and reduced venation, biting and licking mouth parts, a saving or piercing ovipositor, polypod or apodous larvae and pupae generally in cocoons. In the adult frogs the Hymenoptera formed a great percentage of food items. The complete item formed 30.22% and the fragmentary parts formed 19.0% of the total fragmentary stomach contents.

In the juveniles, fthe Hymenoptera was represented among the complete food items by 69.60% and among the fragmentary components it formed 28.75%.

12. Insecta
Coleoptera

The frogs showed a great preference for coleoptera in their diet. Minute to large coleopterans were identified by fore wings modified into elytra, membranous hind wings folded beneath the elytra, large prothorax and biting type of mouth parts. Typical coleopteran larvae were also found. Coleopterans formed 20.68% of the total components found in the stomach. Fragments of the coleoptera also showed an abundance representing 21% of the total fragmentary components.

In the juveniles, complete and fragments of coleopterans formed 7.35% and 9.50% of the total food items respectively.

13. Insect larvae

The stomach contents of the adult frogs, insect larvae comprised 2.29% of the total complete food items and 42% of the total fragmentary food items. In the juveniles fragmentary parts of the larvae formed 15.06% of the total fragmentary food contents.

(b) INTESTINE

The analysis of the food items in the intestine reveals that the food items which were being digested. The contents of the intestine comprised the oligochaetes, arachnids, crustaceans, insects, as complete items or in

fragments. In the contents of the intestine rotifer shells and nematoda were also identified. The nematoda appeared to be parasites and they were found in majority of frogs studied. As none of them was found in the stomach they could not be identified.

In the intestine of the adults, the earthworms comprised 57.14%, the spiders 14.28%, the cockroaches 14.28% and the ants 14.28% as the complete food items. Among fragmentary components of the food, the earthworms formed 13%, oniscus 2%, ants 10%, beetles 13% and insect larvae 50%. About 12% fragmented items due the nematodes.

In the case of the juvenile specimens, spiders and oniscus were seen as complete items, sharing the total percentage comprising 50% each. Among the fragmented parts, shell of rotifer and nematoda were also seen. Rotifers comprised 3.39%, earthworms 3.38%, spiders 3.38%, oniscus 5.08%, prawns 1.69%, bugs and aphids 1.69%, butterflies and moths 3.38%, ants 16.94%, beetles 5.08%, insect larvae 30.50% and nematodes 8.47%.

(8) RECTUM

The analysis of the intact or fragmented food items of the rectum, revealed that these items were not digested. The contents of the rectum comprised the Oligochaetes, Arachnids, Crustaceans, Insects, Insect larvae and Molluscs.

Fig. 3 - Annual occurrence of food items found in the gut of Rana limnocharis Wiegmann during different months of the year.

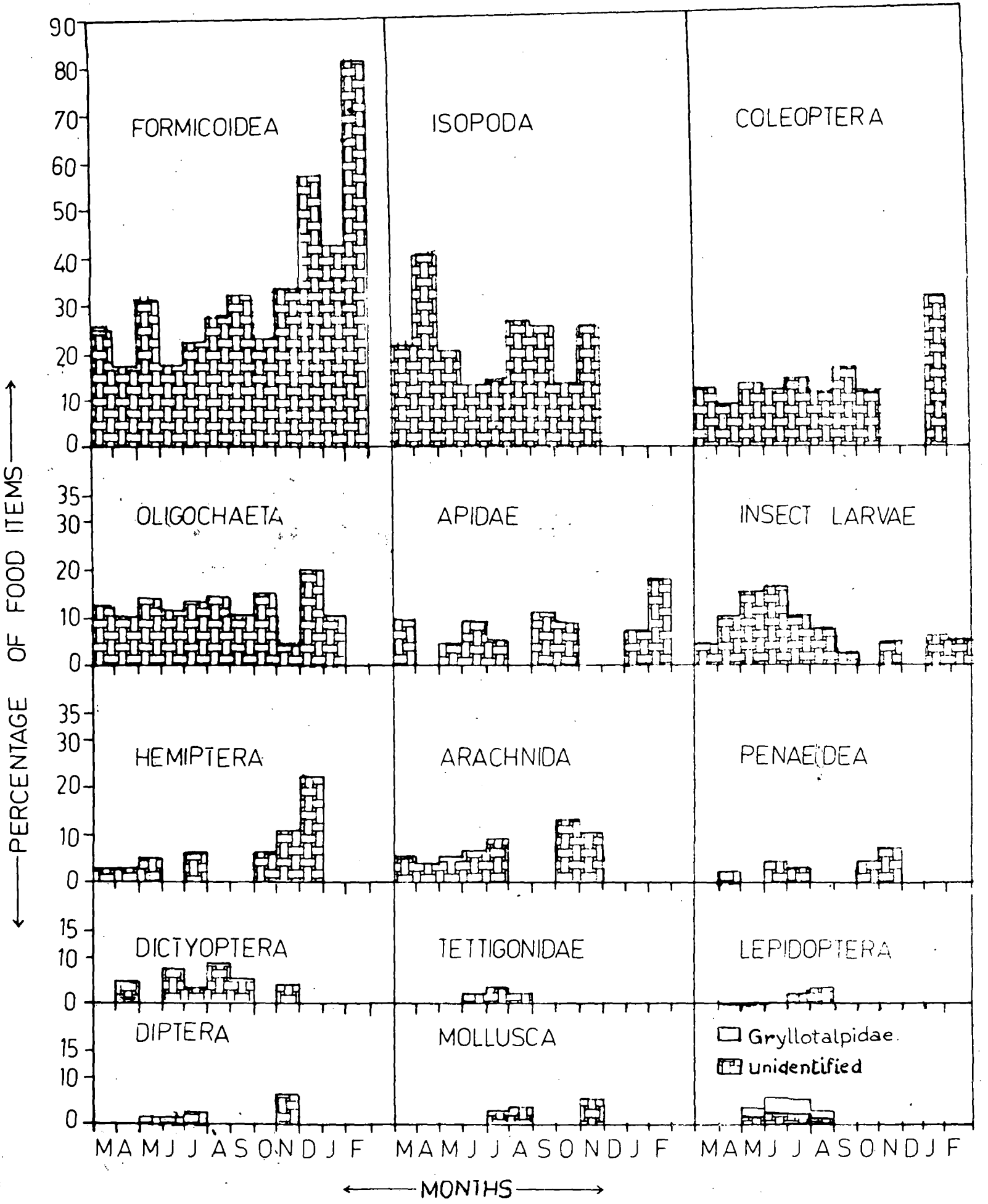
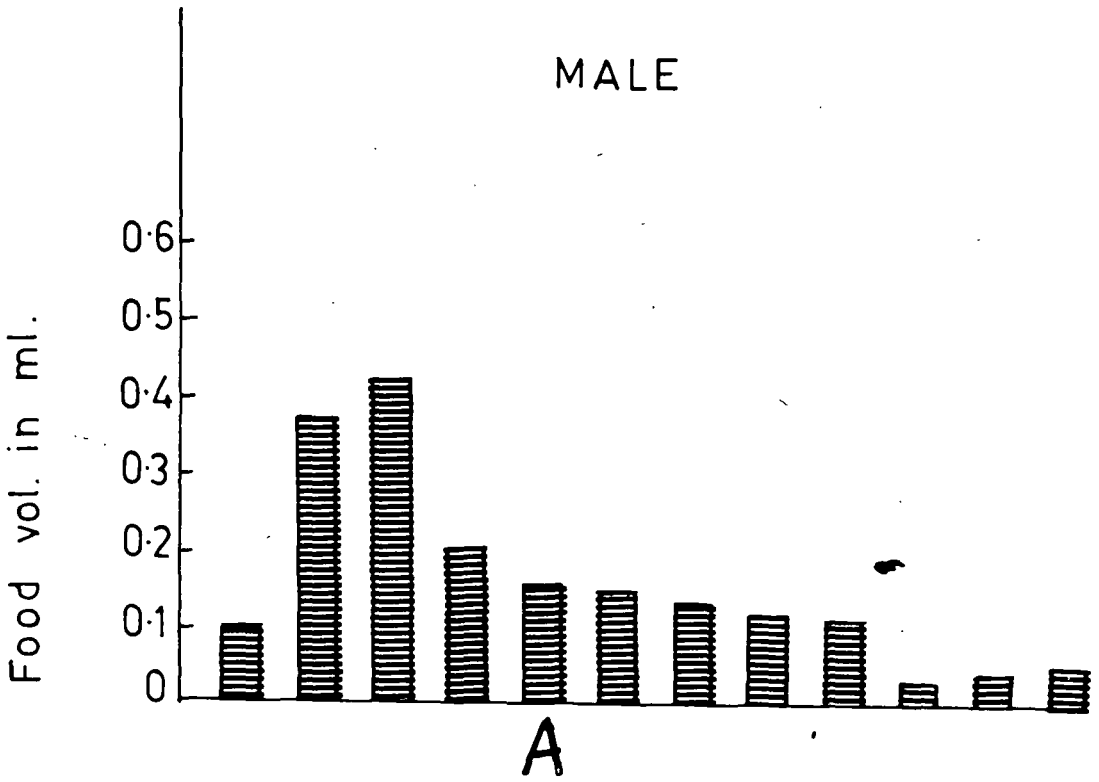
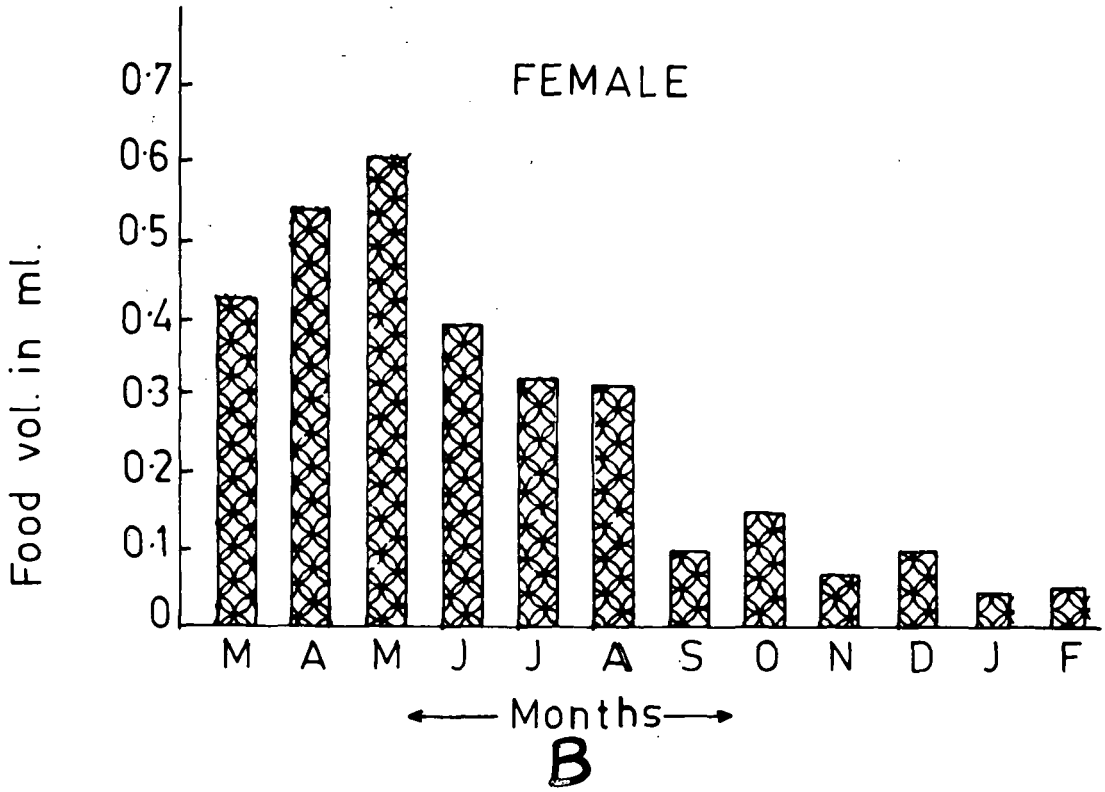

FORMICOIDEA
ISOPODA
COLEOPTERA
OLIGOCHAETA
APIDAE
INSECT LARVAE
HEMIPTERA
ARACHNIDA
PENAEIDEA
DICTYOPTERA
TETTIGONIDAE
LEPIDOPTERA
DIPTERA
MOLLUSCA
Gryllotalpidae
unidentified
PERCENTAGE OF FOOD ITEMS
MONTHS
M A M J J A S O N D J F

Fig 3

Fig. 4 - Volume of food contents found in the gut of adult male and female Rana limnocharis Wiegmann during different months of the year.


MALE
Food vol. in ml.
0·6
0·5
0·4
0·3
0·2
0·1
0

A


FEMALE
Food vol. in ml.
0·7
0·6
0·5
0·4
0·3
0·2
0·1
0
M A M J J A S O N D J F
Months

B

Fig. 4

In the rectum of the adults, among the complete specimens identified, the hymenoptera formed the greatest percentage being 71%; the beetles and insects larvae about 14% each. The fragments were numerous belonging to other groups mentioned above which is not seen among the complete items. Oniscus 1%, cockroaches 1%, bugs and aphids 1%, hymenoptera 1%, beetles 25% and insect larvae 48%. Nematode fragments comprised 13%.

In the juveniles, among complete items, oniscus formed 20%, prawn 20%, bugs and aphids 20% and beetles 40% constituting the highest percentage. Fragmented parts of the food items were numerous in the juvenile frogs also; rotifer shell 1.29%, earthworms 3.38%, spiders 2.69%, oniscus 1.29%, prawn 2.59%, bugs and aphids 2.59%, butterflies and moths 2.59%, mosquito 1.29%, ants 7.78%, beetles 14.28% and other insect larvae 31.16%. Molluscan shell formed about 1.29%. The stones which made up a large volume, 16.88% along with the other fragmented items. They pass out along with the other undigested material.

B. ANALYSIS DURING DIFFERENT MONTHS OF THE YEAR.

The volume of the food intake by adult frogs during different months has been shown in Fig. 4. It was maximum during breeding and minimum during hibernation period. The 12-month data on the analysis of stomach contents has been shown in Fig. 5. The occurrence of different food items fluctuated as described below.

1. Formicoidea

Ants belonging to the group Formicoidea formed the highest percentage of food items taken throughout the year. Their percentage ranged from 25% to 33% during March to November and went up to 57%, 42% and 81% during December, January and February, showing that the frogs take high quantity of these items as food during winter hibernating months.

2. Isopoda

Representatives of Isopoda, i.e. mostly oniscus were found to be the second preference food item fluctuating from 13% to 40% during March to November. They were not recorded in hibernating frogs.

3. Coleoptera

The third preference throughout the year was recorded for Coleopteran beetles. Their amount fluctuated from 9% to 16% during March to October. They were not recorded in samples collected in November and December. In January samples, there was 31% of these beetles. In February samples, they were not found again.

4. Oligochaeta

The earthworms were recorded as fourth preference food items, occuring almost throughout the year except February, fluctuating from 4% to 25% during different months of the year.

5. Apidae

Bees belonging to family Apidae formed the fifth preference food item fluctuating from 4% to 18% in the stomach contents. They were not found in the specimens collected during April, August, November and December.

6. Hemiptera

Hemipterans were recorded in the stomach contents throughout the year except June, August, September, January and February fluctuating from 3% to 22%. The highest percentage observed in December suggests that frogs prefer bugs during hibernation period.

7. Arachnida

Spiders representing Arachnida were recorded in the stomach contents throughout the year except August, September, December, January and February fluctuating from 4% to 14% in different months.

8. Insect larvae

The insect larvae, caterpillars, grubs and maggots were present in the stomach contents almost throughout the year except in the samples examined during October and December. Their percentage fluctuated from 2% to 16%.

9. Penaedae

Small prawns (Macrobrachium species) formed 3% to 7% of the stomach contents during April, June, July,

October and November.

10. Dictyoptera

Cockroaches formed 4% to 8% of the stomach contents during April, June, July, August, September and November.

11. Tettigonidae

Orthoptera represented by tettigonid grasshoppers formed a very small percentage (2% to 4%) of the food items only during breeding season.

12. Lepidoptera

The butterflies and moths also formed a very small percentage of the stomach contents during breeding season, (2% to 3%) in July and August.

13. Diptera

The dipteran flies formed 1% to 2% of the stomach contents during breeding season in May, June, and July and about 6% of the stomach contents in the samples examined during November.

14. Mollusca

Molluscan shells were recorded from the samples examined in July, August and November. During breeding season they formed 2% to 3% and during November they formed 5% of the stomach contents.

15. Gryllotalpidae

Parts of Gryllotalpa were recorded forming 2% to 5% of the stomach contents in some samples during breeding season.

16. Unidentified items

Some fragments present during different months remained unidentified due to their small size.

Important inferences may be drawn from the study of food and feeding habits of Rana limnocharis. (1) It feeds throughout the year including hibernation period (Figs. 3 and 4). (2) It shows food preference in the following order: Formicoidea, Isopoda, Coleoptera, Oligochaeta, Apidae, Hemiptera and Insect larvae among the food items available. (3) Although no data was collected on the size of food items but it was normally observed that larger insects such as gryllotalpa, cockroaches, large grasshoppers were present only in the adults. (4) During breeding season the amount of food taken was much higher specially in the females (Fig. 4B) and during hibernation period the volume of food taken was lowest both in males and as well as in females. (5) Although the frogs show preferences for different items as food, its feeding habits reflect that food items were taken according to their availability, for example insect larvae are more abundant in May, June and July and during this

period they formed a higher percentage of food items present in the stomach. Similarly Dictyopterans, Tettigonids, Lepidopterans, Dipterans and Molluscs were available in the food contents in the months when they were abundantly available. Formicoidae, coleoptera, isopods, oligochaetes, apidae, hemipterans and arachnids were available almost throughout the year and they were found in the stomach contents also, almost throughout the year.

DISCUSSION

PERCENTAGE COMPOSITION OF THE FOOD ITEMS

Most of the workers investigating the food and feeding habits of anurans have concentrated their study on the percentage composition of the food items present in the stomach and other parts of the alimentary canal. Liu and Chen (1932) reported sponges, earthworms, molluscs, shrimps, centipedes, spiders, scorpions, ticks, sowbugs and other crustaceans, Orthoptera, Ephemerids, Odonata, Hemiptera, Homoptera, Coleoptera, Trichoptera, Lepidoptera, Diptera, Hymenoptera and other unidentified insects in the stomach contents of Rana limnocharis and Rana nigromaculata. They reported more affinity of these frogs to insects as compared to other invertebrates. Among insects they reported high percentage of Hymenoptera,

Coleoptera, Homoptera and Diptera. Inger and Marx (1961) investigated extensively on the diet of anurans from Congo, reported that the diet of amphibians depends on the availability of the prey which varies according to different altitudes and different seasons. The size of the prey also limits what can be ingested. Berry (1965), while investigating the food items of Rana limnocharis, Rhacophorus leucomystax, Microhyla butleri, Microhyla hymonsii, Kaloula pulchra, Leptobrachium nigrops found in Singapore, also asserted that diet of anura is dependent on the food items available in the vicinity. He reported a high percentage of Hymenoptera in Microhyla hymonsii, Microhyla butleri and Kaloula pulchra. In Leptobrachium nigrops, orthopterans formed a higher percentage of the food items. Other food items except Dictyoptera were present in low or negligible quantity in the first four species. In 1966, he studied the food items of Amolops larutensis found at Selangor (500-750 ft. above sea level) and reported that ants formed highest percentage of the food. The Ephemeroptera and Coleoptera came in the second category. Pengilley (1970) investigated the food items of Australian anurans Pseudophryne corroboree, Pseudophryne dendyi, Pseudophryne bibroni, Crinia signifera and Hyla verreauxi found in New South Wales (alt. 1040m). He reported a high percentage of Hymenoptera, of which the ants formed the largest proportion in all the species

studied. The next important choice of these frogs was Coleoptera. Houston (1973) investigated the food of Rana temporaria and reported Diptera (Tipulidae) and Lepidoptera to form the highest percentage, whereas phalangid Coleoptera and Gastropoda ranked in the second preference. Elliot and Karunakaran (1974) investigated the feeding habits of Rana cancrivora, which inhabits both brackish and fresh water swamps and ditches in Singapore. He reported great variation in food of this species collected from the two sources: (1) the diet of the specimens collected near the brackish water had a high percentage of crustaceans and crabs; (2) frogs collected near fresh water showed high percentage of insects, of which formicidae formed the highest percentage. Coleoptera, Dipteraand Heteroptera ranked in the second category. Tinsley (1975) reports that diet of frogs can be species specific.

The present study on the food and feeding habits of Rana limnocharis during the active life and breeding period reveals some parallel to the generalised inferences drawn from the investigations described above. For example, Hymenoptera formed the highest percentage approximately 30% of the stomach contents of which ants (formicoidea) formed the main bulk. Oligochaeta formed the second highest percentage approximately 26%. Coleoptera formed the third

preference food items being about 20%, arachnids and isopods (6-8%) formed the fourth preference, and Penaedae and insect larvae about (2-3%) formed the fifth preference food items. Other food items were present only in small quantities. These findings are somewhat different from those of Liu and Chen (1932) and Berry (1965) who reported Coleoptera to form the highest percentage. These variations may be due to different high altitude habitat where the present investigation has been carried out. The monthly analysis throughout the year reveals slightly different picture (Fig. 3). Formicoidea formed the highest percentage throughout the year. Isopoda, Coleoptera, Oligochaeta, Apidae, Insect larvae, Hemiptera, Arachnida and Peneadea were found to occur in varying percentage in order of preference as shown here. Other food items were present only in small percentage and that too according to their availability in different months. The study of food and feeding habits through annual cycle of anurans have not been described by any of the workers except Berry (1966) who has shown seasonal distribution of seven selected prey types ingested by Amolops larutensis (Formicidae, Coleoptera, Orthoptera, Lepidoptera, Diptera, Trichoptera larva and Ephimeroptera nymphs). He did not report any clear seasonal variation in the diet. The present study does reflect a seasonal variation according to

availability of food items. Labanick (1976) found by rank analysis that, prey selection was not as important as prey availability.

Out of the fifteen types food items recorded from the gut of Rana limnocharis, viz. Formicoidea, Isopoda, Coleoptera, Oligochaeta, Apidae, Hemiptera, Arachnida, Insect larvae, Penaedae, Dictyoptera, Tettigonidae, Lepidoptera, Diptera, Mollusca and Gryllotalpidae, only four such as Rotifer shells, Molluscans, shrimp prawns and some insect larvae appear to be aquatic, whereas, all other items were terrestrial in habit. This indicates that this frog is mostly a terrestrial feeder. So far as prey and predator relationship is concerned, larger preys were available in larger specimens examined such as Gryllotalpa, grasshoppers and cockroaches were not recorded from juvenile specimens.

AMOUNT OF FOOD INTAKE

Males

The volume of the food intake in males also shows a fluctuation according to the breeding cycle. Feeding activity continues in males also during hibernation but food volume remains less, measuring 0.03 to 0.07 ml. In March, April and May it was approximately 0.1 to 0.3 ml and afterwards it was 0.1 to 0.16 ml up to November. (Fig. 4A).

Females

The analysis of the volume of the total food intake as calculated on the basis of stomach contents reveals that food intake of females increases enormously (4-4½ times) from hibernation to active breeding period. Data on annual cycle (Fig. 4B) shows that the feeding activity continues during hibernation in December and January also, although the food volume was recorded to be lowest during this period. The volume of the stomach contents during this period was measured to be 0.05 to 0.01 ml, whereas in March and April it was measured to be 0.43 to 0.45 ml. From May onwards there was reduction in the food intake and it remained approximately 0.25 to 0.35 ml up to August. From September onwards it was measured to be 0.1 to 0.15 ml up to November.

FOOD INTAKE AND DIGESTION

On comparing the food items present in the three regions of the alimentary canal it is observed that Oligochaetes, Arachnids, Oniscus, Shrimp prawns, Hymenopterans, Coleopterans and Insect larvae form the main bulk of the food contents in the stomach. Mollusca, ~~Gryllotalpa~~, Tettigonids, Dictyopterans, Hemipterans, Lepidopterans and Dipterans formed a small percentage of food items in the stomach contents. Hymenopterans were present in very large quantity in the juveniles.

In the intestine, Oligochaetes, Arachnids, Oniscus and Insect larvae formed the main bulk. The percentage of Hymenopterans and Coleopterans was less in the intestine and other items were present in still smaller percentage.

In the rectum, Hymenopterans, Coleopterans and Insect larvae formed the main bulk. The percentage of Oniscus and Shrimp prawns was much less. Other items were present in traces.

The presence of food items in varying percentage in stomach, intestine and rectum showed that soft animals such as Oligochaetes and Arachnids are completely digested. Those with hard chitinous exoskeleton Oniscus, Shrimp prawns, Hemipterans, Hymenopterans, Coleopterans and Insect larvae are not fully digested. Their soft parts appear to be partly digested but exoskeleton is not affected.

FROG FOOD AND BIOLOGICAL CONTROL

Frogs have been reported as Biological Control agents. Liu and Chen (1932) while studying the stomach contents of Rana limnocharis and Rana nigromaculata in China calculated the percentage of beneficial and injurious animals eaten and tried to correlate its effect on paddy cultivation in the region from where the frogs were collected. They suggested that the use of frogs as a means for biological control and proposed breeding of

local species to control specific types of crop pests. In the present investigation a small percentage of paddy pests such as grasshoppers and bugs were recorded as food items of Rana limnocharis and it is felt that it can be used as biological control agent for paddy pests. Rana tigrina has also been reported to be a biological control agent for harmful crop pests. (Khan, 1973).

FROGS AND DAMAGE TO PISCICULTURE

The frogs and fresh water fishes breed almost at the same time. There are, however, very few contributions showing the damage caused by frogs to fishes or vice versa. Khonyakina (1961) worked on the feeding habit of Rana ridibunda and Bufo viridis and reported that these species cause damage to pisciculture. It is however, not possible to comment upon the damage caused by Rana limnocharis to pisciculture as it was not worked out in the present investigation. In a preliminary survey the percentage of these frogs was found to be much less in Government Fish Ponds at Shillong, however, none of the specimens examined showed any fish eggs, fragments of fries, fingerlings or adults.

SUMMARY

The results in this chapter deal with 12-month analysis of the food and feeding habits of Rana limnocharis Wiegmann. The size (SVL) of the males ranged from 3.1 to 4.00 cm and the weight from 3.05 to 4.85 gm. The size (SVL) of the females varied from 4.15 to 5.35 cm and the weight from 7.0 to 13.9 gm. The food intake was maximum during the breeding period (males: 0.20 to 0.60 ml and females: 0.25 to 0.80 ml) and minimum during the period of hibernation (males: 0.1 ml and females: 0.8 ml). Gut content analysis during the breeding period revealed that hymenopterans formed about 30% of the stomach contents which was the highest percentage with the ants (Formicoidea) being the main constituent of this group. The oligochaetes formed approximately 26% of the contents being the second highest percentage. The coleopterans (about 20%) formed third largest item of food preference, insect larvae forming 11% were fourth in order of preference, the arachnids and isopods formed about 6% and 8%, being 5th and 6th preferred food items. Other food items such as arthropoda, crustaceans, orthopterans, hemipterans, dipterans, dictyopterans, tettigonids, lepidopterans and miscellaneous insect larvae were found in much less quantities. The monthly analysis revealed Formicoidea forming the highest percentage of the food items throughout the year; isopods, coleopterans,

oligochaetes, apidae, insect larvae, hemipterans, arachnids and peneadea were recorded in varying percentages in that order of preference. The mere presence of food items in the stomach of the frogs during the hibernationa period suggests that they do feed during the hibernation period although in very low quantities. As most of the food items were found to be of terrestrial origin, this frog species can be said to be mostly a terrestrial feeder. Large sizes of prey items were present in the guts of larger frogs while small sized items were seen in the guts of smaller specimens. Oligochaetes, arachnids and soft parts of other items were found fully digested, while the hard chitinous exoskeleton remained undigested. During paddy season a small percentage of paddy pests, such as grasshoppers and bugs were recorded in the gut of this frog suggesting that it had possible role as an agent for biological control.

REFERENCES

Bailey, P. 1976. Food of the marine toad, Bufo marinus and six other species of Skink[frog] in a Cacao plantation in New Britain, Papua New Guinea. Aust. Wildl. Res. 3: 185-188.

Berry, P.Y. 1965. The diet of some Singapore anura. (Amphibia). Proc. Zool. Soc. Lond. 144: 163-174.

Berry, P.Y. 1966. The food and feeding habits of the Torrent frog, Amolops larutensis. J. Zool. Lond. 149: 204-214.

Blackith, R.M. and M.C.D. Speight. 1974. Food and feeding habits of the frog Rana temporaria in bogland habits in the West of Ireland. J. Zool. Lond. 172: 67-79.

Brown, R.L. 1974. Diet and habitat preferences of selected anurans in Southeast Arkansas. Amer. Midl. Nat. 91: 468-473.

Bruggers, R.L. 1973. Food habits of bullfrogs in Northwest Ohio. Ohio. J. Sci. 73(3): 185-188.

Chlodny, J. and T. Mazur. 1969. Food requirements and utilization of food by Rana arvalis Nilss. Ekol. Pol. Ser A. 17(38): 719-733.

Clarke, R.D. 1974. Food habits of Toads, Genus Bufo (Amphibia, Bufonidae). Amer. Midl. Nat. 91(1): 140-147.

Cristea, E., A. Cristea and B. Demetriue. 1972. Observations regarding the food of the green frogs (Rana ridibunda Pall. and Rana esculenta L.) living in the Danube's delta and flooded land . Bul. Crecet. Piscis. 31(3/4): 19-24.

Durant, P. and J.W. Dole. 1974. Food of Atelopus oxyrhynchus (Anura: Atelopodidae) in a Venezuelan cloud forest. Herpetologica. 30(2): 183-187.

Elliot, A.B. and L. Karunakaran. 1974. Diet of Rana cancrivora in freshwater and brackish water environments. J. Zool. Lond. 174: 203-215.

Franz, R. 1970. Food of larval tailed frogs. Bulletin Maryland Herpetological Society. 6(3): 49-51.

Guyetant, R. 1967. Study of the feeding of young frogs in summer . Ann. Sci. Univ. Besancon. Zool. Physiol. Biol. Anim. 3: 69-78.

Heeden, S.E. 1972. Food and feeding behaviour of the mink frog, Rana septentrionalis Baird, in Minnesota. Amer. Midl. Natur. 88(2): 291-300.

Houston, W.W.K. 1973. The food of the common frog, Rana temporaria, on high moorland in Northern England. J. Zool. Lond. 171(2): 153-165.

Inger, R.F. and H. Marx. 1961. The food of amphibians. Exploration du Parc National de l'Upemba. Fascicule. 64: 1-86.

Jenseen, T.A. 1967. Food habits of the green frog, Rana clamitans, before and after metamorphosis. Copeia. (1): 214-218.

Jonssen, T.A. and W.D. Klimstra. 1966. Food habits of the green frog, Rana clamitans, in Southern Illinios. Amer. Midl. Natur. 76(1): 169-182.

Kalusche, D. 1973. Tadpoles as a prey for Rana esculenta . Salamandra. 9(3/4): 164-165.

Khan, M.S. 1973. Food of tiger frog, Rana tigrina Daudin. Biologia. 19(1/2): 93-107.

Khonyakina, Z.P. 1961. Some data on feeding of Rana ridibunda and Bufo viridis in the vicinity of the town Makhachkala. Uch. Zap. Dagestansk. Univ. 7(2): 91-103.

Labanick, G.M. 1976. Prey availability, consumption and selection in the cricket frog, Acris crepitans (Amphibia, Anura, Hylidae). J. Herpetol. 10(4): 293-298.

Liu, Chi-ying and Kan-fan, Chen. 1932. Analysis of stomach contents of two species of frogs (Rana limnocharis and Rana nigromaculata) in the vicinity of Kashing

with special reference to insects. Yb. Bur. Ent. Hangchow (1933)2: 183-191.

Opatrny, E. 1968. The food of our water frogs Rana ridibunda and Rana esculenta . Acta. Univ. Palacki. Olomuc. Fac. Natur. Biol. 28: 133-139.

Pengilley, R.K. 1971. The food of Australian anurans (Amphibia). J. Zool. Lond. 163(1): 93-103.

Smith, M. 1953. The feeding habits of the marsh frogs (Rana ridibunda ridibunda) Brit. J. Herpet. 1: 170-172.

Stewart, M.M. and P. Sandison. 1972. Comparative food habits of sympatric mink frogs, bullfrogs and green frogs. J. Herpetol. 6(3/4): 241-244.

Tinsley, R.C. 1973. Studies on the ecology and systematics of a new species of clawed toad, the genus Xenopus from Western Uganda. J. Zool. Lond. 169: 1-27.

Tinsley, R.C. 1975. The morphology and distribution of Xenopus vestitus (Anura: Pipidae) in Central Africa. J. Zool. Lond. 175: 473-492.

Tucker, J.K. and M.E. Sullivan. 1975. Unsuccessful attempts by bullfrogs to eat toads. Trans. Ill. State. Acad. Sci. 68(2): pp 167.

Tyler, M.J. 1958. On the diet and feeding habits of the edible frog (Rana esculenta Linnæus). J. Zool. Soc. Lond. 131: 583-595.

*Not consulted in original.

Chapter 4

Pituitary - Gonadal cycle.

INTRODUCTION

The role of the pituitary hormones in controlling the somatotrophic and gonadotrophic activities in the vertebrates is now well known. Despite the fact that, there is enormous literature on the amphibian pituitary, there are few contributions which provide us with a clear understanding on the structure and behaviour of the different types of cells of the anterior pituitary in Anura, such as Van Oordt (1963) on Xenopus, Rana and Bufo bufo; Lakshman (1965) on Rana tigrina, Rana cyanophlyctis, Rana hexadactyla, Bufo melanostictus, Uperodon systoma; Rastogi and Chieffi (1970) on Rana esculenta and Zysk (1975) on Rana temporaria. Van Oordt (1963) classified the cells in two types, the acidophils and basophils. He further classified acidophils in 2 types and basophils in 3 types. Lakshman (1965) and Rastogi and Chieffi (1970) investigated the quantitative changes in the cells of pars distalis (anterior pituitary) during annual cycle. They divided the acidophil cells into various types depending upon their staining reactions. Zysk (1975), while investigating the behaviour of cells of the pars distalis of Rana temporaria, described 4 types of cells; acidophils (α cells), 2 types of basophils (β cells and δ cells) and chromophobes. Many workers attempted to correlate the quantitative cytological changes with the physiological

functions of the pituitary and the behaviour of target organs in the amphibians. The basophil cells of the pars distalis have been attributed a direct relationship with ovulation and spermatogenesis (Van Oordt, 1961 and 1965; Lakshman, 1965; Rastogi and Chieffi, 1970). There is a general agreement that the acidophil cells secrete somatotrophins and basophils gonadotrophins. No definite function has been assigned to chromophobes although some workers (Zysk, 1975) believe them to be precursors of basophilic cells.

The present chapter deals with the investigation on the cytological changes of the pituitary gland and the histomorphological changes in the testis and ovary of Rana limnocharis during its annual cycle, with a view to understand the behaviour of the pars distalis, and the different cell types found in it, in relation to the cyclic changes in the gonads and its breeding activity. The investigation on the pars distalis has been restricted to the detailed study of behaviour of 3 major cell types: acidophils (α cells), basophils (β cells) and chromophobes during different phases of the annual cycle. The study of sub-types of cells was not considered necessary during the present investigation. Histological changes in the testis have also been studied in detail throughout the year. As the changes in the ovary were well marked even in the dissection of the females, the investigation on the

ovary has been confined only to the gross morphological and histological changes during different phases of the annual cycle.

REVIEW OF LITERATURE

Work on the pituitary gland in Vertebrates has been reviewed in the recent past by Harris and Donovan (1966) and more recently particularly in Amphibia by Hanke (1974). Among histological and cytological studies, the classification and nomenclature of pituitary cell types have been subjects much debated. Two major types of cells, chromophils and chromophobes have been described by all workers. There is still some confusion over the identification adopted by different investigators. The important contributions, in this connection are following.

As early as in 1892, Schönemann distinguished 2 types of chromophil cells in the pars distalis of human pituitary by using eosin and haematoxylin and named them 'eosinophils' and 'cyanophils'. Using his own formulated stain, Mallory, in 1900, described 2 types of chromophils, acidophils and basophils on the assumption that there were only two types of such cells in the pars distalis. Using Kresofuchsin and Heidenhain's Azan stain, Romeis (1940) did very exhaustive work on human pars distalis. He used the Greek nomenclature for different cell types and

designated ordinary acidophils as alpha cells, those stained orange and present mostly during non-pregnant state as epsilon cells, and those staining similarly as epsilon cells but present during pregnant state as eta cells. McManus (1946) prescribed PAS technique for staining glycoproteins. Using this technique Herlant (1960) identified serous cells and Pearse (1953) identified mucoid cells. Van Oordt (1961) described the gonadotrophic and other cells types in the distal lobe of the pituitary of the common frog, Rana temporaria. Purves (1961) adopted the nomenclature, acidophil and basophil for the 2 types of cells identified by PAS reaction. Due to strong PAS reaction some investigators preferred to call basophils as pasophils. By acid trichrome method Ortman (1956, 1960 and 1961) described 5 chromophil types in Rana pipiens. Van Oordt (1963) worked on Rana, Rana temporaria, Bufo bufo, newts and Salamanders and gave a tentative identification of cell types in amphibians as follows :-

Carminophils = Somatotrophs.

Organgeophils = Prolactin secretors.

Basophils (in order of affinity for Alcian Blue)

1. Purely cyanophil = Thyrotroph.
2. Cyanophil + acidophil inclusions = Folliculotrophs.
3. Amphophil = Interstitiotrophs.

Reyrel (1967), while working upon Pelobates cultrives reported by signalling colourations and histochemical reactions 6 cellular types - 3 acidophilic and 3 basophilic types. Subsequently, besides histological and histochemical, workers also followed flourescent and bioassay techniques. Thus, Mira-Moser (1970) described acidophil I and acidophil II, basophil I, basophil II, basophil III and basophil IV. Kerr (1965), Van Kemende (1974) and Pehlemann (1974) described 2 types of acidophils (acidophil I and acidophil II) and 3 types of basophils (basophil I, basophil II and basophil III). Mira-Moser's basophil I and II correspond to the basophil II and basophil I respectively. Doerr-Schott (1974) described acidophils, basophils and amphophils in amphibians. He reported that acidophils secrete LTH as well as STH. Earlier, Kerr (1965) reported that acidophils I, secrete STH as well as LTH and acidophils II secrete LTH. Van Kemende (1974) attributed secretion of LTH to acidophil I, STH to acidophil II, TSH to basophil I, FSH and LH to basophil II and ACTH to basophil III.

The morphology and development of anuran gonads, has been described in great detail with reference to Rana pipiens by Rugh (1951). There are few detailed contributions on the annual changes in the pituitary gland of Amphibia in relation to gonadal changes. Lakshman (1965) described the structural changes in the pituitary gland of female frogs and toads during different seasons of the year and reported that the

number of acidophilic cells was smallest during the period of egg laying. Rastogi and Chieffi (1970) reported similar phenomena in Rana esculenta. Zysk (1975) gave a detailed account of such changes in the males and females of Rana esculenta. He described 4 types of pituitary cells based on morphological, as well as staining characteristics, acidophil cells (α cells), 2 types of basophil cells (β and δ cells) and chromophobe cells. He described that basophilic cells were numerous soon after hibernation in the I decade of March, acidophilic cells during active period from May to September and chromophobe cells during early breeding period only. The number of chromophobe cells decreases gradually during active egg laying period in the III decade of March. He has correlated these observations with the presence of high level of gonadotrophic hormone during early breeding period and high level of somatotrophic hormone during active land life.

MATERIALS AND METHODS

The pituitary gland and gonads were processed for histological study as follows:

THE PITUITARY GLAND.

The frogs were captured from the streams during paddy season, from the paddy fields of Pologround, Shillong at regular monthly intervals. These animals were taken to the laboratory, decapitated, and the anterior pituitary gland

was taken out and fixed immediately in Bouin's Picro-formol in order to avoid influence of diurnal physiological rhythm in frogs on the result (Lach, 1970). The technique of dissection of the pituitary gland has been described in Chapter V on Induced Breeding.

The pituitaries were fixed in Bouin's fixative, dehydrated in Butanol (normal butyl alcohol) and embedded as per the following procedure :-

1.	Fixation in Bouin's fixative	24 Hours
2.	Washing in 70% alcohol	24 "
3.	Dehydration in 70% alcohol (3/4 part) + Butanol (1/4 part)	1/2 "
4.	Dehydration in 70% alcohol (1/2 part) + Butanol (1/2 part)	1/2 "
5.	Dehydration in 90% alcohol (1/4 part) + Butanol (3/4 part)	1/2 "
6.	Dehydration in Butanol	2 "
7.	Dehydration in fresh Butanol	2 "
8.	Embedding in Butanol (3/4 part) + Paraffin Wax (1/4 part)... ...	1 "
9.	Embedding in Butanol (1/2 part) + Paraffin Wax (1/2 part)... ...	1 "
10.	Embedding in Butanol (1/4 part) + Paraffin Wax (3/4 part).... ...	1 "
11.	Embedding in Molten Paraffin Wax	Over night
12.	Embedding in fresh Molten Paraffin wax ...	2-4 Hours

The paraffin wax blocks were made and microtome sections were cut in sagittal plane of the anterior pituitary,

at thickness of 4 μ. Sections were stained by 2 methods : (1) With Aldehyde-Fuchsin, Orange-G, Haematoxylin, Fast Green and (2) Heidenhain's Azan Stain from which Azocarmine was eliminted. Slidder's method, Barrett's method and PAS techniques described by Disbrey and Rack (1970) did not give satisfactory results. Other techniques could not be attempted as the specific stains could not be obtained. By the use of these two methods the main cell types of the pituitary were differentially stained. By the use of Aldehyde-Fuchsin method, the cytoplasm of the acidophil cells was stained orange and the nucleus reddish violet. The cytoplasm of the basophil cells was stained brown and the nucleus reddish violet. With the Azan stain the cytoplasm of the acidophil cells was stained orange and the nucleus bright orange. For basophil cells, the cytoplasm stained blue and the nucleus reddish blue. The cytoplasm of the chromophobe cells remains unstained or stains very lightly with both the types of stain used. Its nucleus however is stained brightly. The size of the 3 cell types and their nuclei were measured with the help of ocular and stage micrometers. The number of different types of cells per unit area (πr^2 = 2.113 sq. mm) has been calculated on the basis of observations in 5 unit fields under oil immersion (x 1000) of the microscope. Some cells which could not be clearly identified have not been taken into consideration.

Staining technique :

(1) Aldehyde-Fuchsin Method.

1. The sections were deparaffinised in Xylol.
2. Hydrated the sections by passing the slides through 100%, 90%, 70%, 50%, 30% alcohol and then placing them in water.
3. Washed the slides in running water for 2 minutes.
4. Transferred the slides to 70% alcohol.
5. Stained the sections in Aldehyde-Fuchsin for 2 hours.
6. Washed in 70% alcohol.
7. Stained in Ehrlich's Acid Haematoxylin for 5 minutes.
8. Washed in tap water.
9. Stained in 1% aqueous Orange G for 2 minutes.
10. Rinsed in tap water.
11. Dipped in Fast Green stain.
12. Dehydrated in ethyl alcohol.
13. Transferred to Xylol.
14. Mounted in D.P.X.

(2) Heidenhain's Azan Method.

1. Placed the slides in Zylol to deparaffinise the sections.
2. Sections were hydrated.
3. Washed in distilled water.
4. Stained in Aniline Blue, Orange G mixture for 2 hours.
5. Dehydrated the slides in ethyl alcohol and Xylol.
6. Mounted in D.P.X.

THE GONADS

The gonads (testis and ovary) were dissected out at

different periods of the annual cycle from the freshly captured frogs, fixed in small pieces in Bouin's Picro-formol, dehydrated in Butanol in the same way as the pituitary gland, embedded in Paraffin Wax and sectioned at 7 μ thickness. The sections were stained in Haematoxylin and Eosin. Measurements of the various cellular structures were taken with the help of ocular and stage micrometers. The number of sections of the seminiferous tubules were counted under low power magnification of the microscope (each unit area = 7.06 sq. mm). The histological elements of each seminiferous tubule were counted under high magnification (oil immersion) of the microscope (each unit area = 2.113 sq. mm). Diagrams were made with camera lucida and photomicrographs were taken wherever needed.

OBSERVATIONS

THE PITUITARY GLAND

(A) MORPHOLOGY OF THE PITUITARY IN THE VERTEBRATES.

It would be worthwhile to have an idea of gross structure of the pituitary gland before describing its structure in *Rana limnocharis*. In vertebrates, the pituitary gland is located in a small bony fossa, the sella turcica, in the floor of the cranial cavity (☞ Fig. 1). It is connected by a stalk at the base of the brain. Although it is joined to the brain, only part of the pituitary gland develops from the neural ectoderm, as a downgrowth, from

Fig. 1 - Diagrammatic longitudinal section of the pituitary gland in a vertebrate (mammal).

Abbreviations:

Atc - Anterior tuber cinereum.

Ptc - Posterior tuber cinereum.

Inr - Infundibular recess.

Ins - Infundibular stem.

Mde - Median eminence.

Ptb - Pars tuberalis.

Pdt - Pars distalis.

Pim - Pars intermedia.

Pnv - Pars nervosa.

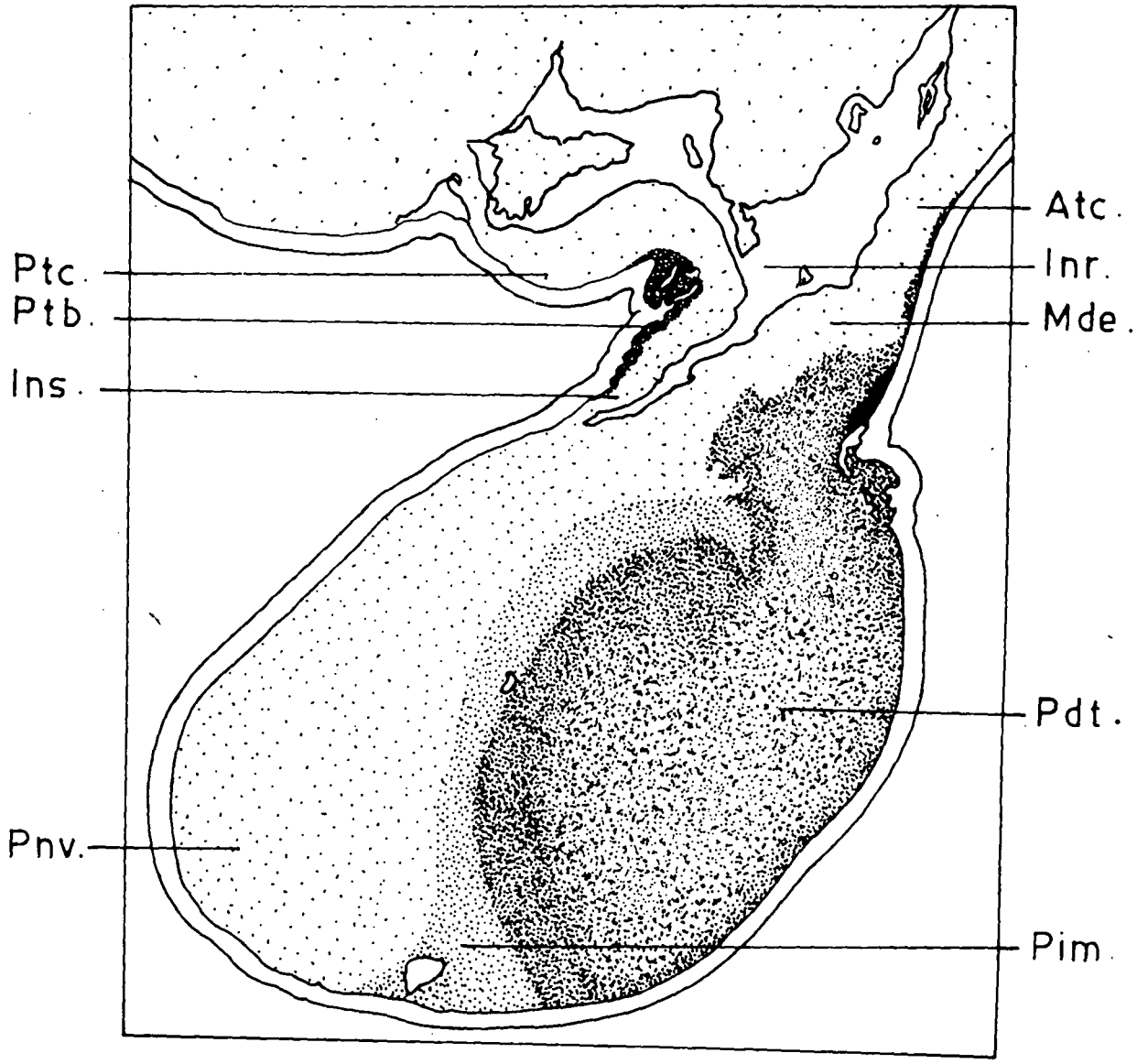


Fig. 1

the floor of the diencephalon. This part is referred to as neurohypophysis. The larger part of the pituitary gland develops from the lining of the future oral ectoderm, the Rathke's pouch, known as the adenohypophysis. The adenohypophysis and the neurohypophysis can be divided in 3 and 2 subdivisions respectively as described below.

Adenohypophysis.

(a) Pars distalis (anterior lobe).
(b) Pars tuberalis.
(c) Pars intermedia (intermediate lobe).

Neurohypophysis.

(a) Pars nervosa (posterior lobe).
(b) Infudibulum.

(B) MORPHOLOGY OF THE PITUITARY GLAND IN RANA LIMNOCHARIS.

The morphology of the pituitary gland in Rana limnocharis, as observed from the ventral side of the skull after removing the palatine bone which forms the floor of the cranium is as follows: (Fig. 2).

Adenohypophysis

The adenohypophysis is situated ventro-caudal to the neurohypophysis. It is composed of the pars distalis, pars intermedia and pars tuberalis.

(a) Pars distalis (Fig. 3)

It forms the posterior lobe of the pituitary and is broad, often broader than long, bean shaped, pinkish, located

Fig. 2 - Ventral view of the brain of Rana limnocharis Wiegmann showing the position of the pituitary.

Abbreviations:

Olf. Bulb - Olfactory bulb	Nst - Nostril
Tel - Telencephalon	Med - Medulla
Eyeb - Eye ball	Cbl - Cerebellum
Optc - Optic tectum	Pty - Pituitary
Opch - Optic chiasma	Dien- Diencephalon

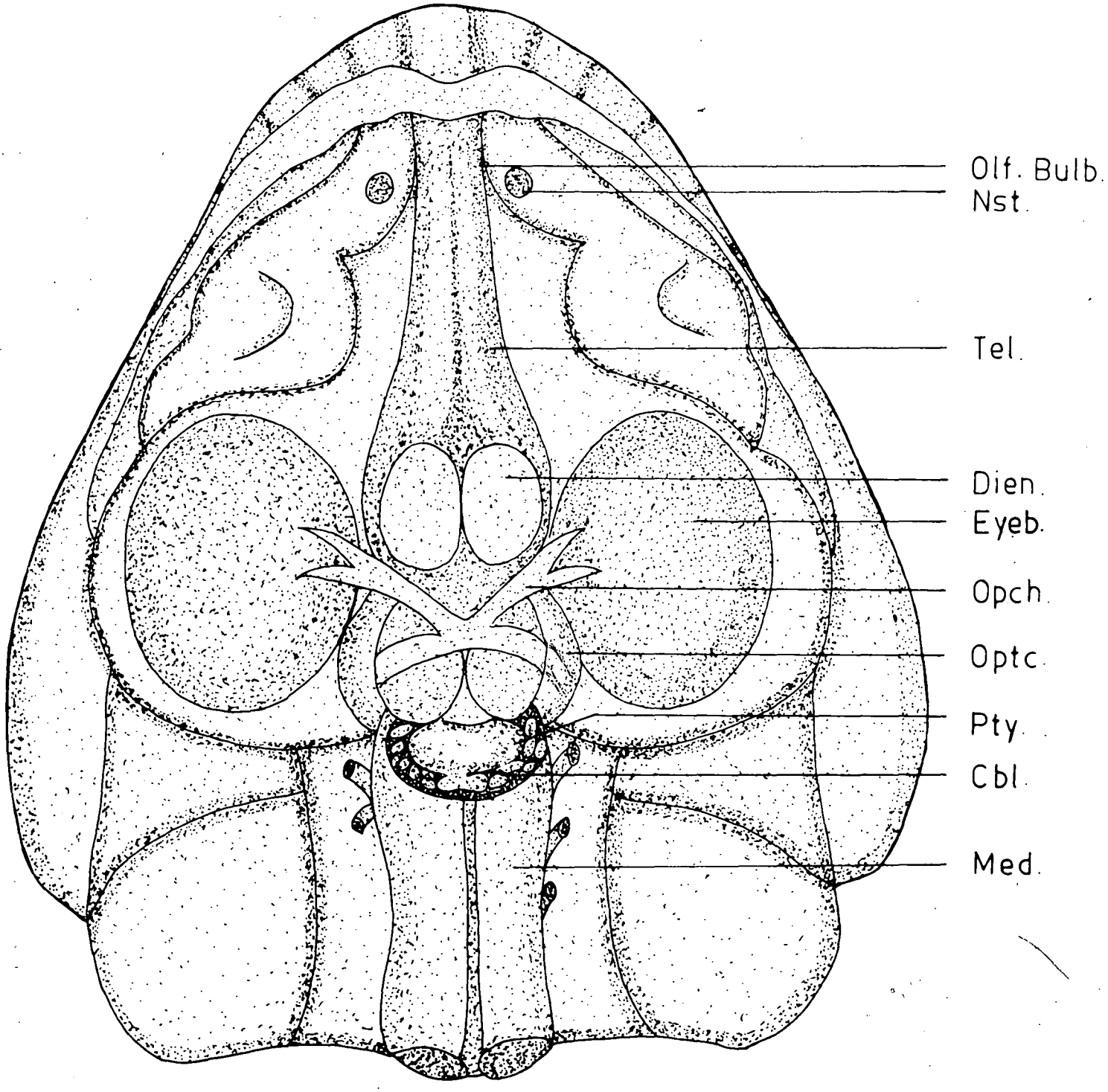


Fig 2

0·2 cm

Fig. 3 - Ventral view of the brain showing the pituitary of Rana limnocharis Wiegmann.

Abbreviations:

Prc - Preoptic recess
Opch - Optic chiasma
Pdt - Pars distalis
Eyeb - Eye ball
Ptb - Pars tuberalis

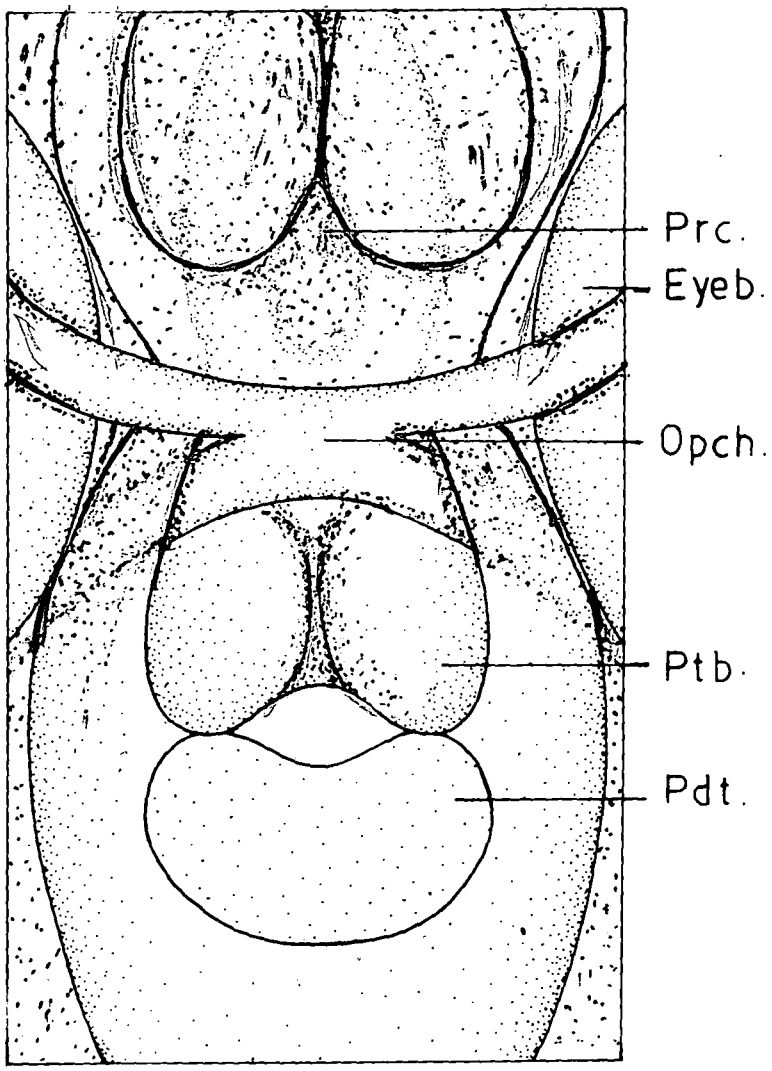


Fig. 3

0.1cm

transversely posterior to optic chiasma. It is continuous with the pars intermedia along its antero-dorsal margin and is attached by means of connective tissue. It remains connected to the median eminence with its antero-ventral extremity. It is commonly referred to as anterior pituitary gland.

(b) Pars intermedia

It covers the postero-ventral aspect of the neural lobe and the two together form a transverse bar, the 'neuro-intermediate lobe' which is broader than the pars distalis.

(c) Pars tuberalis

It develops from the compact hypophyseal anlage, as a pair of epithelial tongue, which extend rostrally along the surface of the hypothalamus. As such, in the dissection of the brain it cannot be distinctly marked out from the hypothalamus.

Neurohypophysis

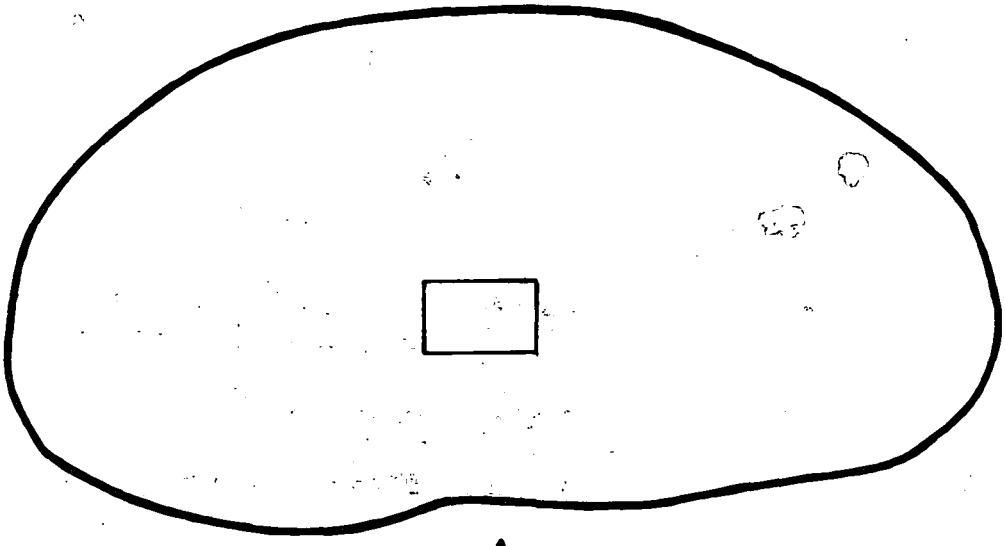
The neurohypophysis is not as well developed as the adenohypophysis. The wide succus infundibuli grows out as a pair of primary branches forming a transverse bar, situated over the anterior part of the hypophysis. The posterior wall of this bar proliferates to form the neural lobe. It stores a great amount of neuro-secretory material. The median eminence is situated antero-ventrally to the neural lobe, in contact with the antero-ventral end of the pars distalis.

Fig. 4 A - Outline diagram of the pituitary gland of Rana limnocharis Wiegmann.

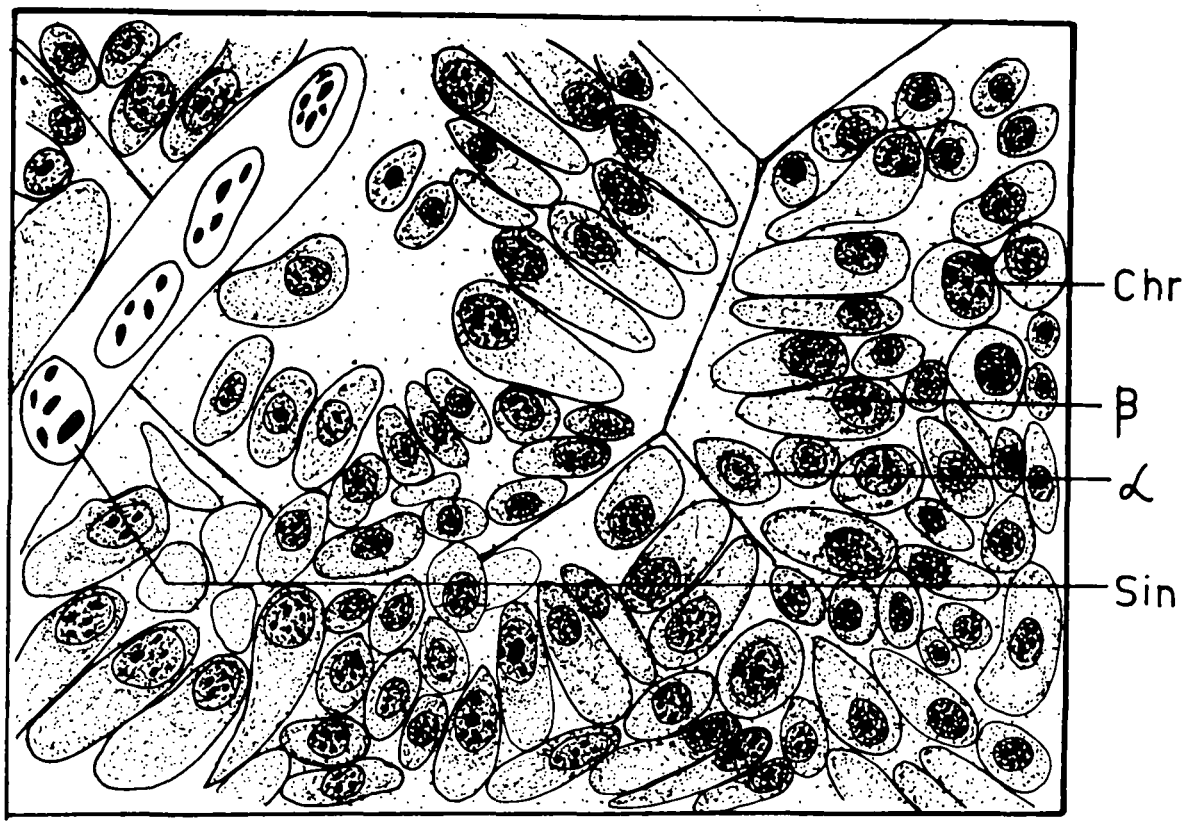
Fig. 4 B - A central portion of the cross-section of the pituitary gland of Rana limnocharis Wiegmann.

Abbreviations:

Chr - Chromophobe cells
Sin - Sinusoids
β - Basophil cells
ℭ - Acidophil cells



A



B

Fig 4

HISTOLOGICAL STRUCTURE

In the present investigation, the histological changes in the pars distalis (anterior pituitary) have been studied. As observed in the cross sections (Fig. 4A and B), the cells of the pars distalis are mainly arranged in cords, more or less in radiating pattern, between which are large bore of capillaries. Some cells appear to be in clusters, other in twisted cords and still others form small, well defined follicles, the lumina of which contain colloid. There is a very light meshwork of connective tissue fibre, entwined around the cells and capillaries.

There is a clear distinction in the arrangement of cells between the central and peripheral regions of the anterior pituitary. At the peripheral region, the cell cords are densely packed separated by relatively thin connective tissue and a plexus of capillaries. In the central region, the cells are less compact and larger, surrounded by large capillaries and more loosely arranged connective tissue. Three major types of cells were easily identified by the stains used : (a) Acidophil or α cells (b) Basophil or β cells and (3) Chromophobes.

(a) Acidophil or α cells.

The acidophil or α cells are spherical or ellipsoidal in shape with a centric nucleus. Cytoplasm is strongly acidophilic and contains dense granules. These cells usually

Fig. 5 - Diagrammatic representation of the distribution of acidophil, basophil and chromophobe cells in the pituitary gland of Rana limnocharis Wiegmann.

A - Distribution of cells during pre-breeding period.

B - Distribution of cells during breeding period.

C - Distribution of cells during post-breeding period.

D - Distribution of cells during hibernation period.

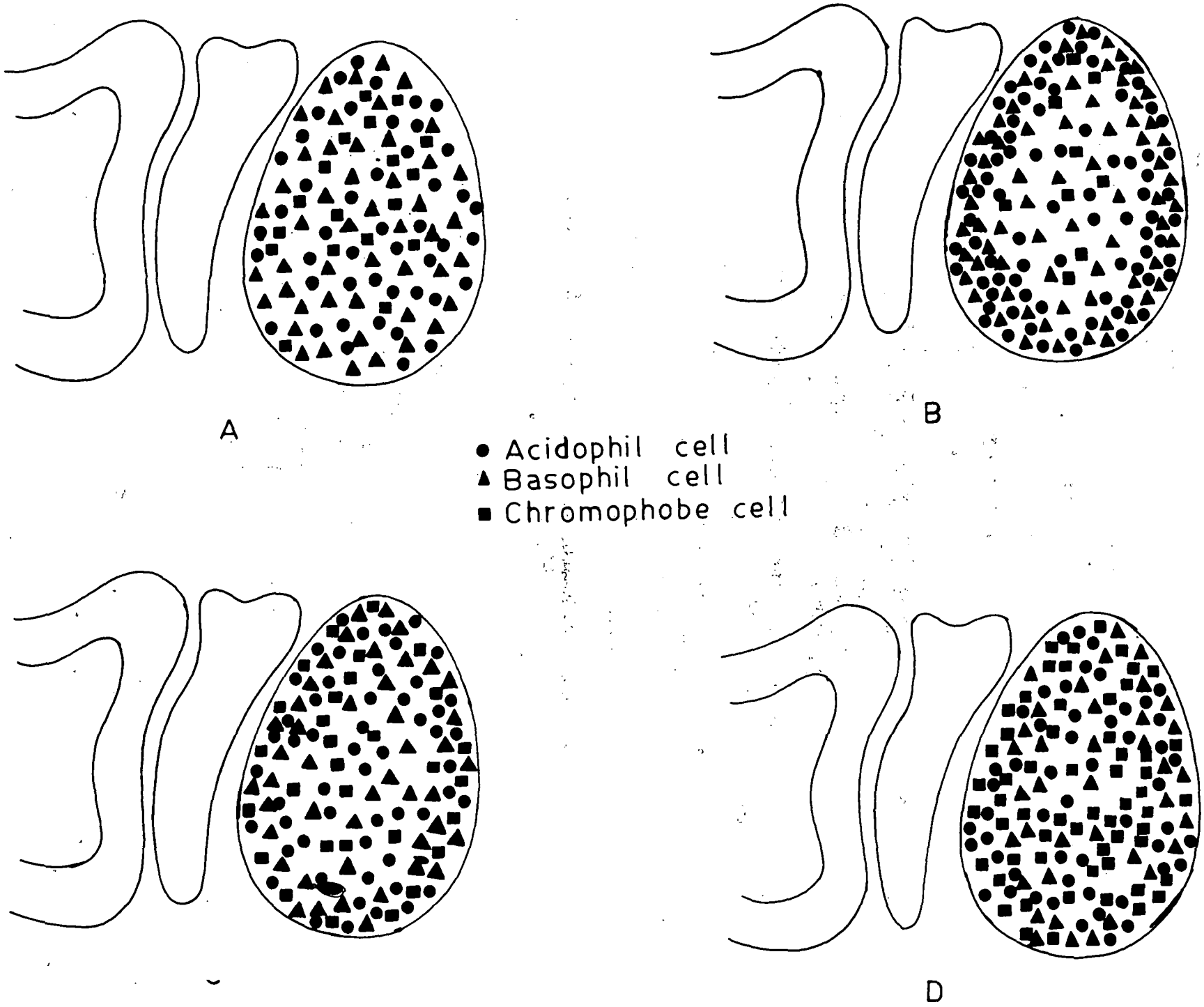


Fig.5

occupy central place in the lobule and are said to be responsible for producing somatotrophic hormone (STH).

(b) Basophil or β cells.

The basophil or β cells are the largest cells found in the gland. They are spherical, elongated, oval or angular in shape. They have eccentric nucleus. Cytoplasm is strongly basophilic. Their arrangement in the lobules is like columnar epithelium. Granulation varies depending upon the secretory phase of the cells. They are said to be responsible for producing thyrotrophic hormone (TSH), follicle-stimulating hormone (FSH) and luteinizing hormone (LH).

(c) Chromophobes

The chromophobes are small or large spherical cells, with eccentric nucleus. The cytoplasm stains poorly with both acidic and basic dyes. Thus, the cytoplasm is either poorly stained or not stained at all. It may or may not possess granules in the cytoplasm depending upon the secretory condition of the cell. They occur singly or in clusters and are not clearly organized in the follicles.

(C) CHANGES IN THE BEHAVIOUR OF DIFFERENT TYPES OF CELLS DURING ANNUAL CYCLE.

The behaviour of acidophil cells, basophil cells and chromophobes was studied during the following different periods of their annual cycle (Table I and II; Fig. 5A, B, C and D).

TABLE I

Average number of different types of cells per unit area (2.113 sq. mm) observed in the pituitary gland of Rana limnocharis during annual cycle.

Month of Investigation	Acidophil or α cell	Basophil or β cell	Chromophobe cell
January	3.88	5.77	3.40
February	6.43	9.65	2.93
March	3.21	11.35	1.32
April	13.90	29.05	4.16
May	13.44	23.28	1.60
June	22.19	24.04	1.18
July	21.95	37.08	1.98
August	18.55	23.56	2.74
September	15.99	23.89	3.02
October	8.51	7.19	2.65
November	5.48	5.77	2.36
December	4.63	5.20	2.08

TABLE II

Average cell size and nuclear diameter (mm) of different types of cells in the pituitary gland of Rana limnocharis during annual cycle.

Month of investigation	Acidophil or α cell			Basophil or β cell			Chromophobe cell		
	Cell size		Nuclear diameter	Cell size		Nuclear diameter	Cell size		Nuclear diameter
	Length Width	Length Width		Length Width	Length Width		Length Width	Length Width	
Jan.	0.12 - 0.10	0.10-0.14 0.08-0.12	0.07	0.18 - 0.11	0.16-0.20 0.10-0.14	0.08	0.22 - 0.16	0.20-0.28 0.14-0.20	0.10
Feb.	0.12-- 0.10	0.12-0.14 0.10-0.12	0.09	0.16-- 0.13	0.16-0.18 0.12-0.14	0.09	0.16 - 0.14	0.16-0.18 0.12-0.16	0.10
Marc.	0.13 - 0.12	0.12-0.16 0.10-0.14	0.08	0.13 - 0.10	0.10-0.12 0.10-0.12	0.08	0.11 - 0.10	0.08-0.16 0.08-0.14	0.07
Apr.	0.16 - 0.15	0.16-0.20 0.12-0.20	0.08	0.23 - 0.13	0.20-0.26 0.12-0.16	0.11	0.10 - 0.10	0.10-0.12 0.10-0.12	0.08
May	0.13 - 0.12	0.10-0.16 0.10-0.16	0.07	0.18 - 0.13	0.18-0.20 0.12-0.16	0.09	0.12 - 0.12	0.10-0.14 0.10-0.14	0.08
Jun.	0.12 - 0.12	0.12-0.14 0.10-0.14	0.09	0.24 - 0.13	0.20-0.30 0.10-0.16	0.09	0.14 - 0.14	0.12-0.16 0.14-0.16	0.11
Jul.	0.15 - 0.14	0.10-0.17 0.10-0.24	0.08	0.22-- 0.13	0.14-0.28 0.08-0.12	0.07	0.10 - 0.09	0.10-0.10 0.08-0.10	0.07
Aug.	0.17 - 0.14	0.16-0.20 0.12-0.20	0.08	0.17 - 0.16	0.16-0.18 0.12-0.14	0.09	0.10 - 0.08	0.10-0.08 0.06-0.08	0.08
Sep.	0.16-0.13	0.14-0.18 0.12-0.16	0.08	0.17 - 0.14	0.16-0.18 0.12-0.16	0.08	0.09 - 0.09	0.08-0.09 0.06-0.10	0.08
Oct.	0.10 - 0.16	0.10-0.10 0.12-0.16	0.08	0.18 - 0.10	0.16-0.22 0.10-0.12	0.08	0.10 - 0.10	0.10-0.12 0.10-0.12	0.08
Nov.	0.22 - 0.08	0.20-0.24 0.08-0.10	0.06	0.22 - 0.10	0.20-0.26 0.08-0.12	0.08	0.12 - 0.10	0.10-0.12 0.08-0.10	0.08
Dec.	0.17 - 0.12	0.12-0.20 0.10-0.18	0.08	0.14 - 0.13	0.12-0.20 0.10-0.14	0.08	0.15 - 0.16	0.14-0.16 0.12-0.20	0.06

Plate I A - A portion of the cross section of the pituitary gland of Rana limnocharis Wiegmann during pre-breeding period. (x 1000)

Plate I B - A portion of the cross section of the pituitary gland of Rana limnocharis Wiegmann during breeding period. (x 1000)

Plate I C - A portion of the cross section of the central region of the pituitary gland of Rana limnocharis Wiegmann during post-breeding period. (x 1000)

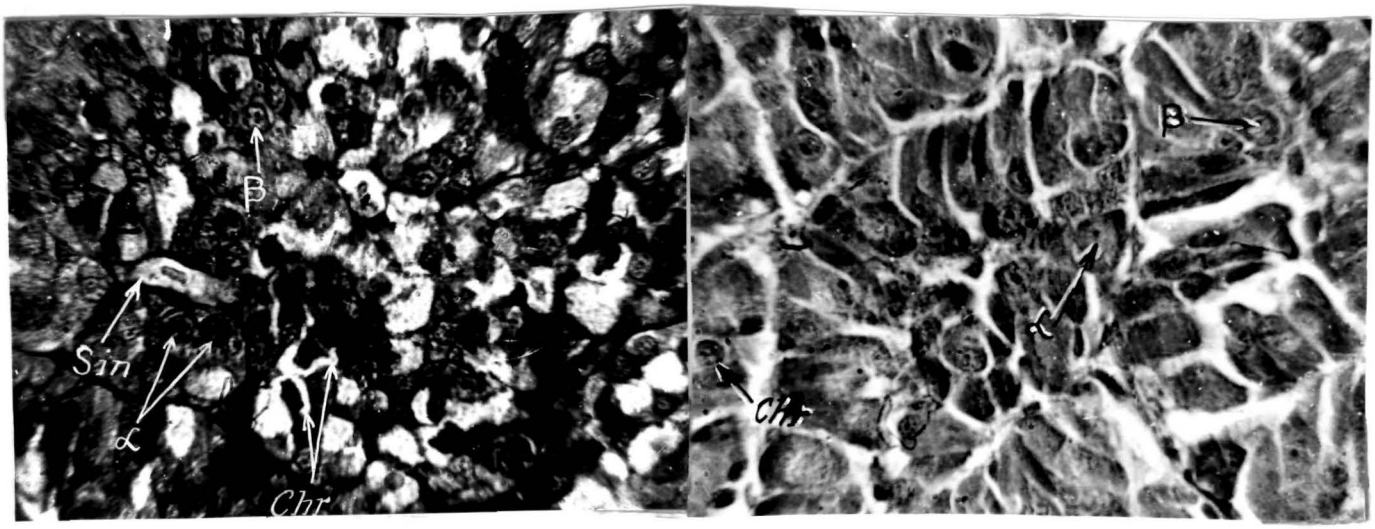
Plate I D - A portion of the cross section of the peripheral region of the pituitary gland of Rana limnocharis Wiegmann during post-breeding period. (x 1000)

Abbreviations :

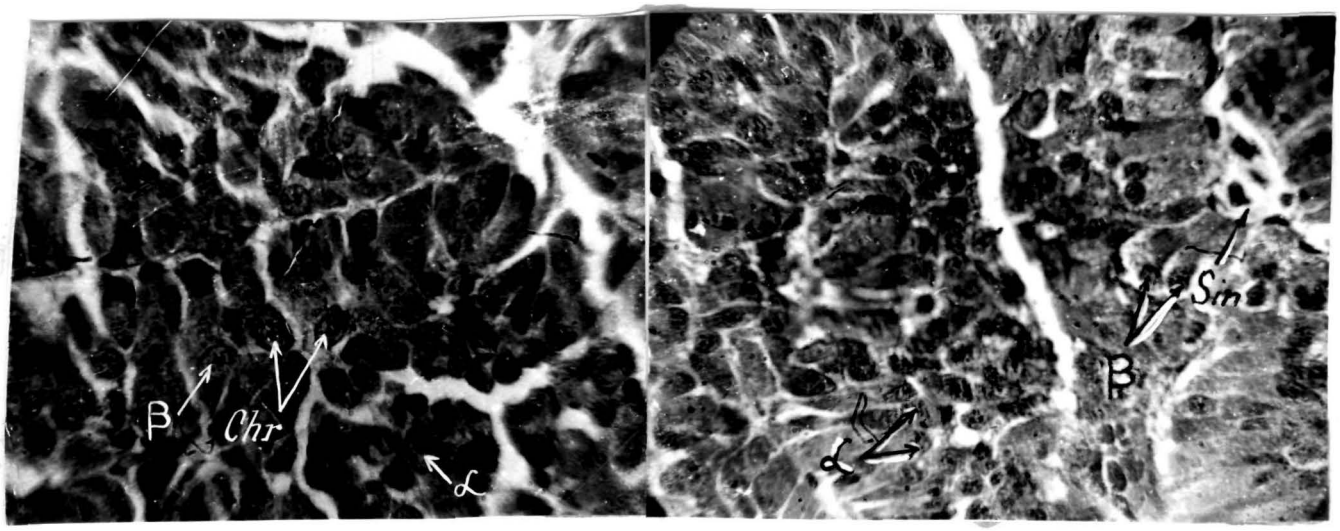
Chr - Chromophobe cells Sin - Sinusoids

α - Acidophil cells β - Basophil cells

Plate I



A B



C D

1. Pre-breeding Period (Late March).
2. Breeding Period (April, May, June, July and August.
3. Post-breeding Period (September and October).
4. Hibernation Period (November, December, January and February).

1. Pre-breeding Period.

The pre-breeding period is the period starting from the time of emergence from hibernation to the time when amplexus and spawning begin. During the short duration of the pre-breeding period the distinction between central and peripheral regions as seen during post-breeding and early hibernation periods, was not observed. In comparison to the months of hibernation the cells were compact in arrangement; and few intercellular spaces were seen during pre-breeding period. There was distinct granulation in the cytoplasm of the acidophil as well as the basophil cells during this period (Plate IA). The description of the three types of cells observed during pre-breeding period is as follows :

(a) Acidophil cells : The average number of acidophil cells per unit area was 3.21. Their size was noted to be approximately 0.13 X 0.12 mm. Their nuclear diameter was measured to be approximately 0.08 mm.

(b) Basophil cells : The average number of basophil cells per unit area, approximate cell size and nuclear diameter were noted to be 11.35, 0.13 X 0.10 mm and 0.08 mm respectively.

(c) Chromophobe cells : The average number of chromophobe cells per unit area, approximate cell size and nuclear diameter were noted to be 1.32, 0.11 X 0.10 mm and 0.07 mm respectively.

2. Breeding Period.

During breeding period the central region was observed to be loosely packed with cells in comparison to the peripheral region. There was heavy granulation in the cytoplasm of the acidophil as well as the basophil cells, and their nuclei had highly dispersed chromatin indicating apparently the highly active phase of these cells. The chromophobe cells were few in number and smaller in size indicating that they were less active during this period (Plate IB). The details of the behaviour of the three types cells observed during breeding period is as follows :

(a) Acidophil cells : Gradual increase was noted in the average number of acidophil cells per unit area during different months of the breeding period. It was recorded to be 13.9, 13.44, 22.19, 21.95 and 18.55 during April, May, June, July and August respectively. Increase in the cell size was noted in the months of April and August; in May and June, the size was less, the approximate cell size being 0.16 X 0.15 mm, 0.13 X 0.12 mm, 0.12 X 0.12 mm, 0.15 X 0.14 mm, 0.17 X 0.14 mm in April, May, June, July and August respectively. The nuclear diameter also increased,

being greatest in the month of June. It was measured to be approximately 0.08 mm, 0.07 mm, 0.09 mm, 0.08 mm and 0.08 mm in April, May, June, July and August respectively.

(b) Basophil cells : The number of the basophil cells during the breeding period was large in comparison to that observed during other periods of the annual cycle. During the breeding period the largest number of these cells was observed in July. From August onwards there was a decline in their number, the average number per unit area being 29.05, 23.28, 24.04, 37.08 and 23.56 in the months of April, May, June, July and August respectively. The cell size increased in the months of April, May, June and then showed a gradual increase during the rest of the breeding season. The approximate cell size was noted to be 0.23 X 0.13 mm, 0.18 X 0.13 mm, 0.24 X 0.13 mm, 0.22 X 0.13 mm, 0.17 X 0.16 mm in April, May, June, July and August respectively. The nuclear diameter was measured to be approximately 0.11 mm, 0.09 mm, 0.09 mm, 0.07 mm and 0.09 mm in the months of April, May, June, July and August respectively.

(c) Chromophobe cells : The number of these cells was much less in comparison to that observed during other months of the year. It started increasing from July and August. Their average number per unit area was observed as 4.16, 1.60, 1.18, 1.93 and 2.74 during April, May, June, July and August respectively. The cell size increased from April to June,

and decreased during July and August. It measured approximately 0.10 X 0.10 mm, 0.12 X 0.12 mm, 0.14 X 0.14 mm, 0.10 X 0.09 mm, 0.10 X 0.08 mm in April, May, June, July and August respectively. The nuclear diameter was found to be greatest in June, in comparison to the other months of the year. It measured approximately 0.08 mm, 0.08 mm, 0.11 mm, 0.07 mm and 0.08 mm in April, May, June, July and August respectively.

3. Post-breeding Period.

The distinction between the cells of central and peripheral region was more distinct during the post-breeding season. The cytoplasm was more dense in comparison to the earlier months of the year. The number and activity of the acidophil and basophil cells gradually decreased, whereas, that of chromophobe cells increased. (Plate I C and D).

(a) Acidophil cells : The average number of acidophil cells per unit area decreased to 15.99 in September and 8.51 in October. The size of cells also became smaller being 0.16 X 0.13 mm in September and 0.10 X 0.16 mm in October. The nuclear diameter remains approximately 0.08 mm during both the months.

(b) Basophil cells : The average number of basophil cells per unit area also decreased being 23.09 in September and 7.19 in October. The size of cells decreases in September and October, being approximately 0.17 X 0.14 mm in September

and 0.18 X 0.10 mm in October. The nuclear diameter remains approximately 0.08 mm for September and October.

(c) Chromophobe cells : The average number of cells per unit area in comparison to that of the breeding season was observed to be more, being 3.02 in September and 2.65 in October. From this period onwards gradual increase in the number of these cells was observed reaching maximum during hibernation period. The cell size also showed similar behaviour increasing approximately to 0.09 X 0.09 mm in September and 0.10 X 0.10 in October. The nuclear diameter remained similar during both the months, being 0.08 mm in September as well as in October.

4. Hibernation Period.

In the early phase of the hibernation period the anterior pituitary showed a clear distinction between its peripheral and central regions. The cells in the peripheral region were more compactly arranged in comparison to those in the central region. The cell boundaries were not very distinct and few sections of the blood vessels were observed in between them. During later phase of the hibernation period, the sections of the anterior pituitary showed a compact cellular arrangement and distinction between its central and peripheral region was not so clearly observed. Intercellular spaces in between the cells were observed. Blood vessels were large and chromophobe cells appeared to

Plate II E - A portion of the cross section of the pituitary gland of Rana limnocharis Wiegmann during early hibernation period. (x 1000)

Plate II F - A portion of the cross section of the pituitary gland of Rana limnocharis Wiegmann during late hibernation period. (x 1000)

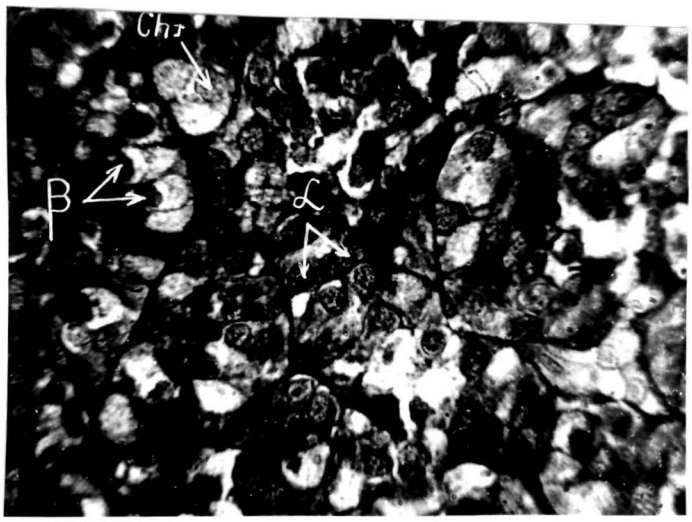
Abbreviations :

Chr - Chromophobe cells Sin - Sinusoids

α - Acidophil cells β - Basophil cells

Plate II

E



F

be more active than acidophil and basophil cells. The details of the behaviour of the three types of cells during hibernation period are given below. (Plate II E and F).

(a) Acidophil cells : The number of acidophil started decreasing in the early months of hibernation. In the last phase of the period of hibernation their number gradually rises. In comparison to other months, the number of acidophil cells during the whole period of hibernation remained less. Their number decreased from November to January. From February their number again increased. The average number of acidophils per unit area was 5.48, 4.63, 3.88 and 6.43 during November, December, January and February respectively. The acidophil cells were also larger during hibernation period. From November onwards the cell size increased being approximately 0.22 X 0.08 mm, 0.17 X 0.12 mm, 0.12 X 0.10 mm and 0.12 X 0.10 mm in the months of November, December, January and February respectively. The nuclear diameter of the acidophil cells during hibernation period was measured to be approximately 0.06 mm, 0.08 mm, 0.07 mm and 0.09 mm during November, December, January and February respectively.

(b) Basophil cells : The basophil cells like the acidophil cells were less in number during the period of hibernation as compared to other months of the annual cycle. A decrease in their number was observed from November to February, the average number per unit area being 5.77, 5.20, 5.77 and 9.65

during the months of November, December, January and February respectively. The cell size was also smaller during the period of hibernation in comparison to other months of the year. However, in comparison to the acidophil cell, the cell size of the basophil cell was larger. The size of basophil cells started decreasing from November, being approximately 0.22 X 0.10 mm, 0.14 X 0.13 mm, 0.18 X 0.11 mm, 0.16 X 0.13 mm in the months of November, December, January and February respectively. There was hardly any fluctuation in the nuclear diameter. It was measured to be approximately 0.08 mm, 0.08 mm, 0.08 mm and 0.09 mm in the months of November, December, January and February respectively.

(c) Chromophobe cells : The average number of chromophobe cells per unit area was more than the number of acidophil and basophil cells during the period of hibernation in comparison to the other months of the year. The number gradually increased from November and reached maximum in the month of January and then a decline in their number was noted, being 2.36, 2.08, 3.40 and 2.93 in the months of November, December, January and February respectively. The cell size was also maximum during the months of hibernation. The size increased from November onwards, reached maximum in January being approximately 0.12 X 0.10 mm, 0.15 X 0.16 mm, 0.22 X 0.16 mm, 0.16 X 0.14 mm during the months of November, December, January and February respectively. The nuclear

diameter showed fluctuation to some extent being more during the months of January and February. It was measured approximately to be 0.08 mm, 0.06 mm, 0.10 mm and 0.10 mm from November to February.

The above observations reveals that the average number and cellular size of the acidophil and basophil cells was maximum during breeding period and minimum during hibernation period, whereas, the average number and cellular size for the chromophobe cells was maximum during hibernation period and minimum during the breeding period.

THE TESTIS

(A) MORPHOLOGY OF THE TESTIS. (Plate III G)

The testis of Rana limnocharis are paired, whitish ovoid bodies, lying ventral to and near the anterior end of each kidney. They are suspended to the dorsally placed kidney by a double fold of peritoneum known as mesorchium. The mesentry surrounds each testis and is continuous with the peritoneal epithelium which covers the ventral face of each kidney and lines the entire body cavity. The spermatozoa are produced in the seminiferous tubules, which in a section of the testis are observed as closely packed, oval-shaped sacs, separated from each other by the partitions of supporting tissue, known as interstitial tissue. The interstitial tissue is continuous with the covering of the testis known as tunica albuginea.

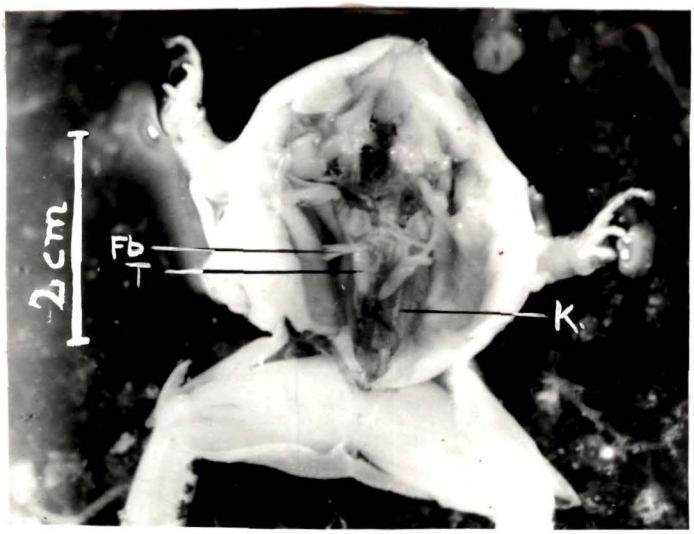
Plate III G - Dissection of male Rana limnocharis Wiegmann showing testis.

Plate III H - Dissection of female Rana limnocharis Wiegmann showing ovary and oviduct.

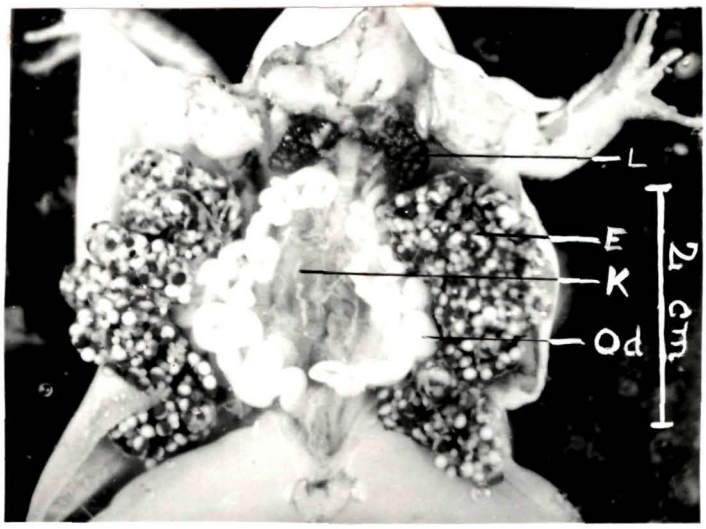
Abbreviations :

Fb - Fat body	T - Testis
K - Kidney	L - Lung
E - Egg	Od - Oviduct

Plate III



G



H

HISTOLOGICAL STRUCTURE.

The detailed histological structure of the seminiferous tubules of Rana limnocharis is as follows :

1. Seminiferous tubule : The wall of the convoluted seminiferous tubule consists of (i) an outer capsule or tunica propria of fibro elastic connective tissue and flattened fibroblasts, which closely invests the tubule (ii) a basement membrane and (iii) a lining of complex stratified epithelium which consists of two kinds of cells: (a) the sertoli cells and (b) the spermatogenic cells.

(a) Sertoli cells : These are tall, irregularly arranged columnar cells, that extend from the basal lamina to the lumen. Their sides are marked by uneven showing pits and depressions, into which fit the adjoining cells. The location of the nucleus varies in different sertoli cells, from the basal position to position located at a considerable distance from the basal lamina. The nucleus is ovoid and pale staining with finely dispersed chromatin, and it usually contains one or more nucleoli.

(b) Spermatogenic cells : They lie in between the sertoli cells in an orderly manner, with 4 to 8 layers occupying the space between the basal lamina and the lumen, representing all stages of differentiation viz., primitive germ cell or spermatogonia, primary spermatocyte, secondary spermatocyte, spermatids and spermatozoa according to the sexual maturity.

Primitive germ cells or spermatogonia : These cells are located directly inside the basal lamina and give rise to spermatozoa ultimately. They are spherical or cuboidal in shape. Nucleus is spherical with granular chromatin. They divide so as to maintain their own number and to give rise to the cells that differentiate into spermatocytes.

Primary spermatocyte : These cells are formed from the innermost layer of the spermatogonia. They are larger cells, larger than the spermatogonia and have large vesicular nuclei showing variable appearance of chromatin depending upon the functional state of the cell. It may be in the form of either elongated spiremes or condensed chromosomes, preparatory to cell division.

Secondary spermatocytes : These cell arise from primary spermatocytes, each primary spermatocyte giving rise to 2 secondary spermatocytes. They are smaller than the primary spermatocytes and lie internal to them.

Spermatids : These cells are derived by the division of the secondary spermatocytes and are located adjoining the lumen of the tubule. They are easily recognized by their small size and location. These cells form the last generation in the spermatogenic process. They undergo no further division, but by profound changes in their structure they become transformed into mature spermatozoa. Groups of maturing spermatids can be seen in close association with the sertoli cells.

Spermatozoa : The spermatozoa consist of a distinct head and an elongated tail. These are slender, motile and flagellate bodies. Nearly mature spermatozoa are frequently observed with their heads in close association with the cytoplasmic processes of the sertoli cells and their tails extending out into the lumen of the tubule. Mature spermatozoa are seen in bundles at the centre of the lumen.

(B) CHANGES IN THE TESTIS DURING ANNUAL CYCLE.

Histological sections of the testes prepared regularly at different periods of the annual cycle were studied. The data on the measurements of various histological structure (mean of 5 observations in each case) has been given in Tables III and IV. Following major changes were observed in the testes through different periods of the annual cycle.

1. Pre-breeding Period.

Immediately after the period of hibernation, the frogs enter a short pre-breeding period in the month of March. During this period the average number of sections of seminiferous tubules under low power of the microscopic field was 9.40. The details of different structural elements of the seminiferous tubules studied under high power of microscopic field were as follows. (Fig. 6; Plate IV J. & K)

(a) Sertoli cells : The average number of sertoli cells were 2.55 per unit area. The approximate cell and nuclear diameter of these cells was 0.67 mm and 0.07 mm respectively.

Fig. 6 - A portion of the cross-section of the testis during pre-breeding period.

Fig. 7 - A portion of the cross-section of the testis during breeding period.

Abbreviations:

Ser	-	Sertoli cell
Sptg	-	Spermatogonia
P.Spte	-	Primary spermatocyte
S.Spte	-	Secondary spermatocyte
Sptd	-	Spermatid
Spte	-	Spermatozoa

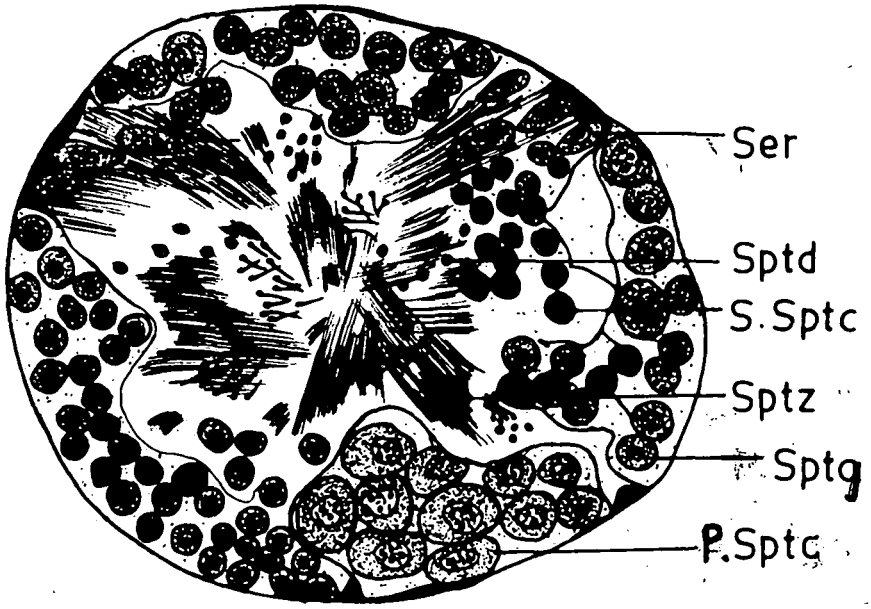

Ser
Sptd
S.Sptc
Sptz
Sptg
P.Sptc
0.2 mm

Fig 6

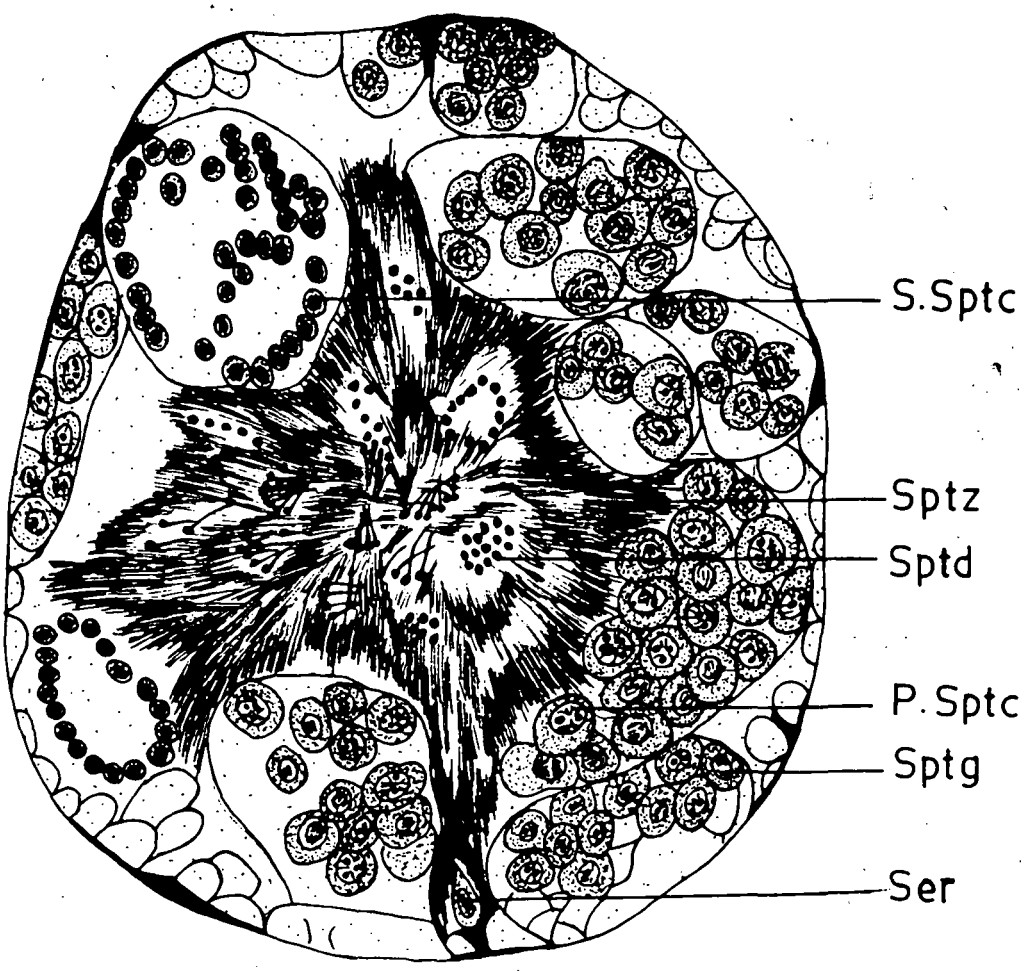

S.Sptc
Sptz
Sptd
P.Sptc
Sptg
Ser
0.2 mm

Fig 7

Plate IV I - A portion of the cross section of the testis of Rana limnocharis Wiegmann during late hibernation period. (x 450)

Plate IV J - A portion of the cross section of the testis of Rana limnocharis Wiegmann during early pre-breeding period. (x 450)

Plate IV K - A portion of the cross section of the testis of Rana limnocharis Wiegmann during late pre-breeding period. (x 450)

Plate IV L - A portion of the cross section of the testis of Rana limnocharis Wiegmann during breeding period. (x 450)

Abbreviations :

Ser - Sertoli cell

Sptg - Spermatogonia

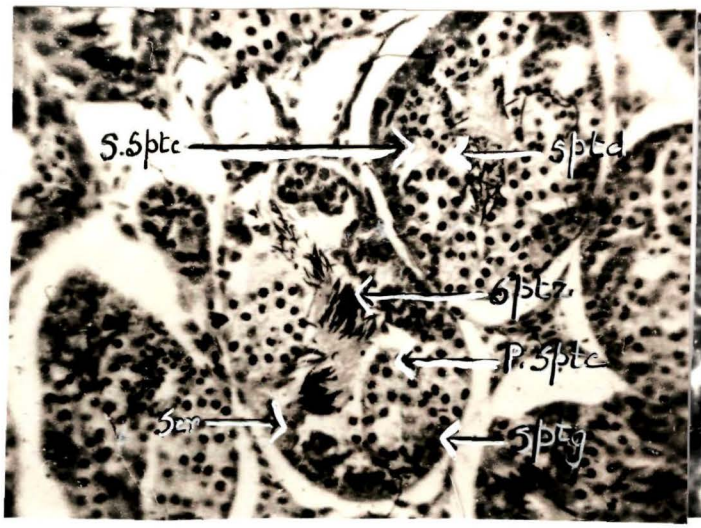
P.Sptc - Primary spermatocyte

S.Sptc - Secondary spermatocyte

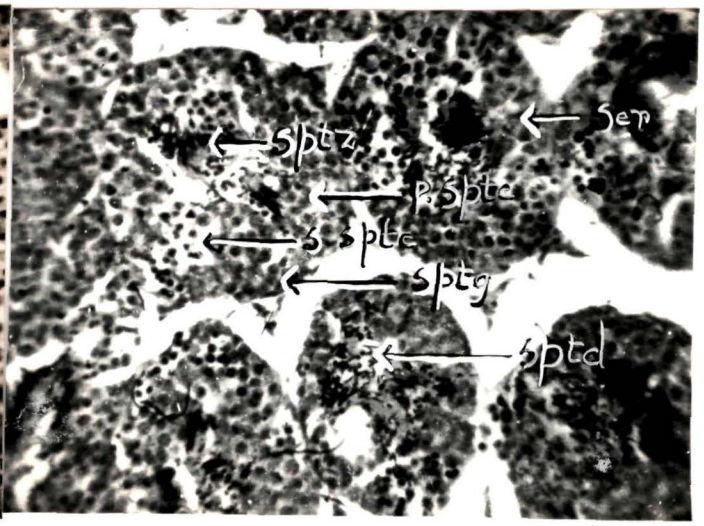
Sptd - Spermatid

Sptz - Spermatozoa

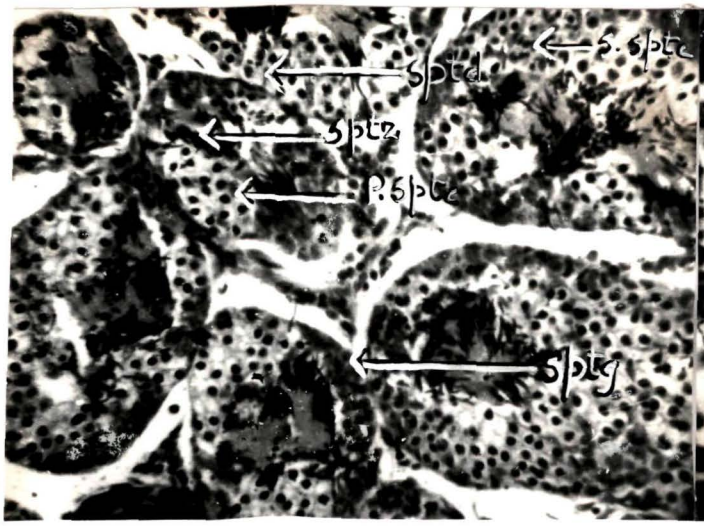
Plate IV



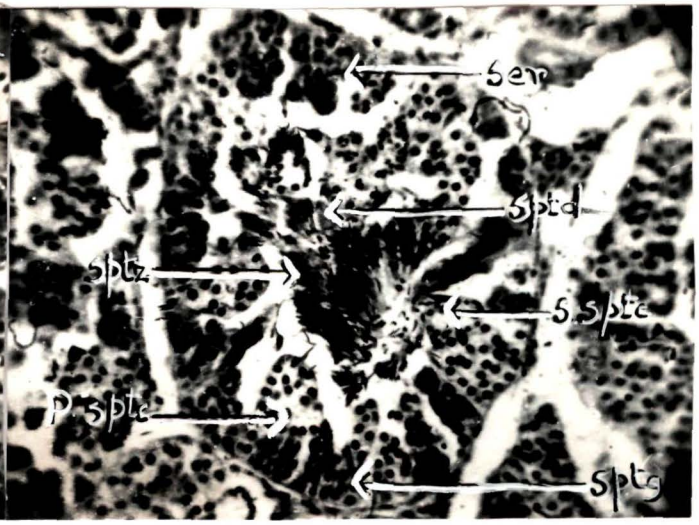
I



J



K



L

TABLE III

Average number of Seminiferous tubules per unit area (7.06 sq. mm) and the average number of different structural elements of the Seminiferous tubules per unit area (2.113 sq. mm) observed in the testes of Rana limnocharis during annual cycle.

Month of investigation	Seminiferous tubules	Sertoli cell	Spermat-ogonial cell	Primary sperma-tocyte	Second-ary sp-ermato-cyte	Sperma-tids	Sperma-tozoa
January	7.36	2.27	10.69	23.94	15.52	6.62	129
February	7.56	2.36	7.85	14.86	16.75	8.51	203
March	9.40	2.55	6.62	13.25	17.79	8.61	296.6
April	6.11	1.41	8.80	11.45	17.88	11.54	308.4
May	5.69	1.79	8.32	9.74	14.86	20.44	445.4
June	5.04	1.41	3.69	9.46	5.48	22.40	453.6
July	4.84	1.32	3.40	9.65	6.90	18.93	481.4
August	4.27	1.32	9.46	9.74	11.73	16.63	424.5
September	4.53	1.04	19.87	14.43	12.77	7.09	302
October	4.58	1.32	19.97	14.67	13.06	6.53	298
November	8.98	1.89	14.19	16.37	13.62	4.63	75
December	11.81	1.98	13.72	17.41	14.67	6.53	99

TABLE IV

Average cell and nuclear diameter (mm) of the structural elements of the seminiferous tubules of Rana limnocharis during annual cycle.

Month of investigation.	Sertoli cell		Spermatogonial cell		Primary spermatocyte		Secondary spermatocyte		Spermatids	
	Cell diameter	Nuclear diameter.	Cell diameter	Nuclear diameter	Cell diameter	Nuclear diameter	Cell diameter	Nuclear diameter	Cell diameter	Nuclear diameter
January	0.68	0.10	1.03	0.12	0.93	0.09	0.54	0.07	0.10	0.04
February	0.80	0.26	0.64	0.10	0.90	0.10	0.60	0.07	0.12	0.04
March	0.67	0.07	0.53	0.08	0.79	0.08	0.60	0.07	0.24	0.05
April	0.64	0.07	0.51	0.07	0.60	0.08	0.69	0.06	0.12	0.04
May	0.62	0.05	0.95	0.08	0.58	0.07	0.58	0.06	0.11	0.04
June	0.57	0.06	0.56	0.07	0.48	0.07	0.25	0.04	0.08	0.04
July	0.50	0.05	0.54	0.08	0.62	0.08	0.33	0.06	0.09	0.04
August	0.48	0.07	0.51	0.10	0.79	0.08	0.40	0.06	0.08	0.03
September	0.50	0.07	0.92	0.10	0.80	0.08	0.40	0.05	0.06	0.04
October	0.53	0.07	0.96	0.12	0.82	0.07	0.44	0.06	0.07	0.05
November	0.60	0.07	1.46	0.13	0.84	0.09	0.45	0.06	0.06	0.04
December	0.66	0.07	1.04	0.11	0.87	0.10	0.50	0.06	0;08	0.04

(b) Spermatogonial cells : The average number of spermatogonial cells was 6.62 per unit area and the cell and nuclear diameter of these cells was noted to be approximately 0.53 mm and 0.08 mm respectively.

(b) Primary spermatocytes : The average number of primary spermatocytes per unit area was 13.25 and their cell and nuclear diameter was approximately 0.79 mm and 0.08 mm respectively.

(d) Secondary spermatocytes : The average number was 17.70 per unit area and their cell and nuclear diameter was approximately 0.90 mm and 0.07 mm respectively.

(e) Spermatids : The average number per unit area was 8.61 and their cell and nuclear diameter was approximately 0.24 mm and 0.05 mm respectively.

(f) Spermatozoa : The average number of spermatozoa per unit area was 296.6.

2. Breeding Period.

The active breeding period was observed during April, May, June, July and August. The average number of sections of the seminiferous tubules per unit area was 6.11 in April, 5.69 in May, 5.04 in June, 4.84 in July and 4.27 in August. Various cellular types of each tubule showed following changes. (Fig. 7; Plate IV-L; V-M, N, O & P).

Plate V M,N,O and P - Portions of the cross section of the testis of Rana limnocharis Wiegmann during different months of the breeding period. (x 450)

Abbreviations :

Ser - Sertoli cell

Sptg - Spermatogonia

P.Spte - Primary spermatocyte

S.Spte - Secondary spermatocyte

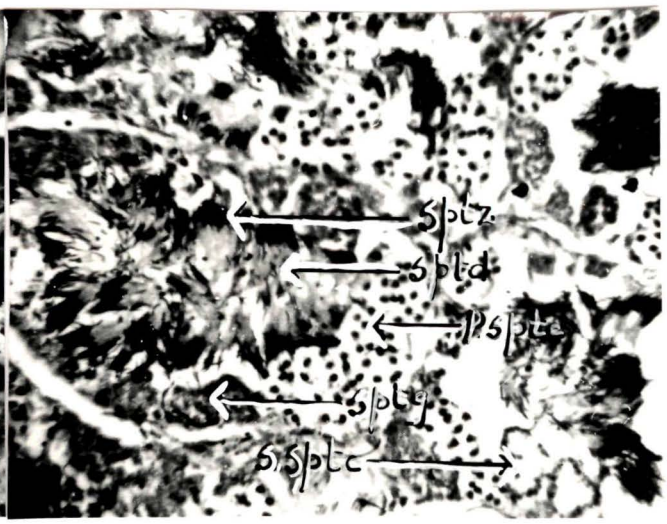
Sptd - Spermatid

Sptz - Spermatozoa

Plate V


Sptz
Sen
Sptd
S.Spte
P.Spte
Sptg

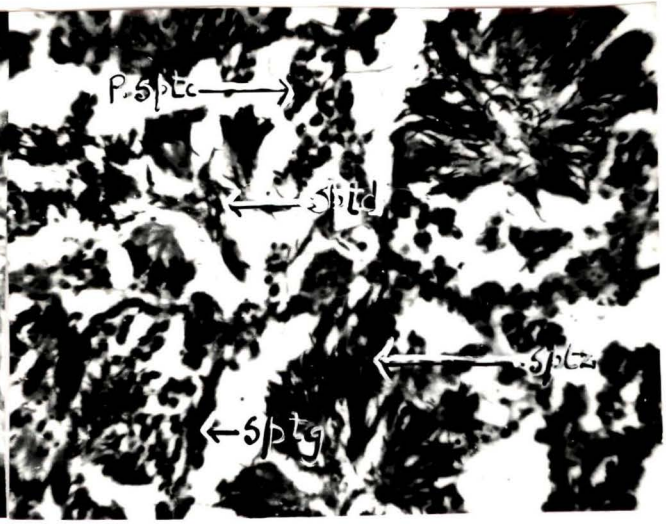
M


Sptz
Sptd
P.Spte
Sptg
S.Spte

N


Sptg
P.Spte
S.Spte
Sptd
Sptz

O


P.Spte
Sptd
Sptz
Sptg

P

(a) Sertoli cells : The average number of sertoli cells per unit area was observed to be 1.41, 1.79, 1.41, 1.32 and 1.32 in the months of May, June, July and August respectively. The diameter of these cells during these months was approximately 0.64 mm, 0.62 mm, 0.57 mm, 0.50 mm and 0.48 mm; and their nuclear diameter was approximately 0.07 mm, 0.05 mm, 0.06 mm, 0.05 mm and 0.07 mm respectively.

(b) Spermatogonial cells : The average number of spermatogonial cells per unit area was 8.80, 8.32, 3.69, 3.40 and 9.46; their diameter was approximately 0.51 mm, 0.95 mm, 0.56 mm, 0.54 mm and 0.51 mm; and their nuclear diameter was approximately 0.07 mm, 0.08 mm, 0.07 mm, 0.08 mm and 0.10 mm during April, May, June, July and August respectively.

(c) Primary spermatocytes : The average number of primary spermatocytes per unit area was 11.45, 9.74, 9.46, 9.65, 9.74; their diameter was approximately 0.60 mm, 0.58 mm, 0.48 mm, 0.62 mm and 0.79 mm; and their nuclear diameter was approximately noted to be 0.08 mm, 0.07 mm, 0.07 mm, 0.08 mm and 0.08 mm during April, May, June, July and August respectively.

(d) Secondary spermatocytes : The average number of secondary spermatocyte per unit area was 17.88, 14.86, 5.48, 6.90 and 11.73; their diameter was approximately 0.69 mm, 0.58 mm, 0.25 mm, 0.33 mm, 0.40 mm during April, May, June, July and August respectively; and their nuclear diameter was approx-

imately 0.06 mm throughout the breeding period, except for the month of June when it measured to be approximately 0.04 mm.

(e) Spermatids : The average number of spermatids per unit area was 11.54, 20.44, 22.40, 18.93 and 16.63; their diameter was approximately 0.12 mm, 0.11 mm, 0.08 mm, 0.09 mm, 0.08 mm and their nuclear diameter was approximately 0.04 mm, 0.04 mm, 0.04 mm, 0.04 mm and 0.03 mm for April, May, June, July and August respectively.

(f) Spermatozoa : The average number of spermatozoa per unit area was observed to be 308.4, 445.4, 453.6, 481.4 and 424.5 during April, May, June, July and August respectively.

3. Post-breeding Period.

During post-breeding period, the average number of the sections of seminiferous tubules per unit area was 4.53 and 4.59 in the months of September and October respectively. The following changes in the histological structures of each tubule were observed during this period. (Fig. 8; Plate VI Q).

(a) Sertoli cells : The average number of sertoli cells was 1.04 and 1.32 per unit area during September and October, their diameter being approximatelt 0.50 mm and 0.53 mm respectively. Their nuclear diameter remained approximately 0.07 mm for both the months,

Fig. 8 - A portion of the cross-section of the testis during post-breeding period.

Fig. 9 - A portion of the cross-section of the testis during hibernation period.

Abbreviations:

Ser	-	Sertoli cell
Sptg	-	Spermatogonia
P.Sptc	-	Primary spermatocyte
S.Sptc	-	Secondary spermatocyte
Sptd	-	Spermatid
Sptz	-	Spermatozoa

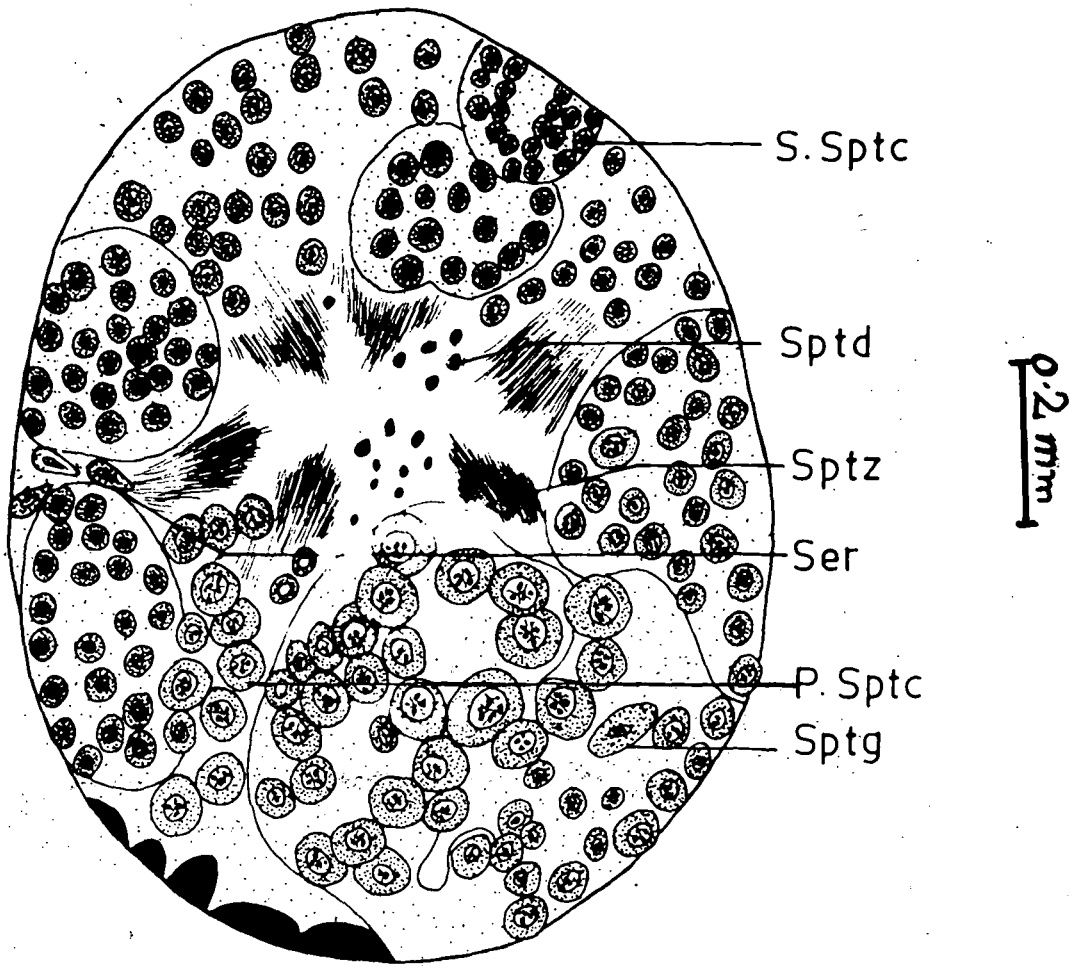

S. Sptc
Sptd
Sptz
Ser
P. Sptc
Sptg
0·2 mm

Fig 8

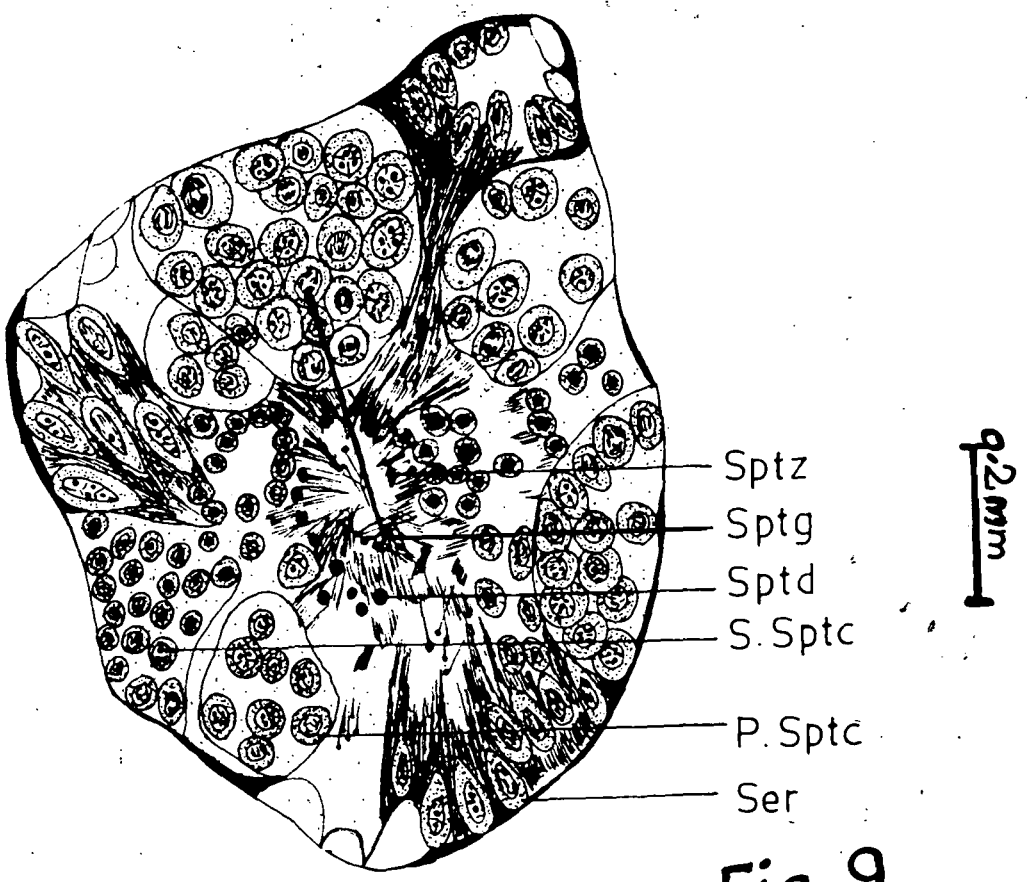

Sptz
Sptg
Sptd
S. Sptc
P. Sptc
Ser
0·2 mm

Fig 9

(b) Spermatogonial cells : The average number of spermatogonial cells per unit area was 19.87, 19.97; their diameter approximately was 0.92 mm and 0.96 mm, and their nuclear diameter approximately was 0.10 mm and 0.12 mm during the months of September and October respectively.

(c) Primary spermatocytes : The average number of primary spermatocytes per unit area was 14.43 and 14.67; their diameter was approximately 0.80 mm and 0.82 mm and their nuclear diameter was approximately 0.08 mm and 0.07 mm during September and October respectively.

(d) Secondary spermatocytes : The average number of secondary spermatocytes per unit area was 12.77 and 13.06; their diameter was approximately 0.40 mm and 0.44 mm and their nuclear diameter was approximately 0.065 mm and 0.06 mm during September and October.

(e) Spermatids : The average number of spermatids per unit area was 7.09 and 6.53; their diameter was approximately 0.06 mm and 0.07 mm and their nuclear diameter was approximately 0.04 mm and 0.05 mm for September and October respectively.

(f) Spermatozoa : The average number of spermatozoa per unit area was 302 and 298 for the months of September and October respectively.

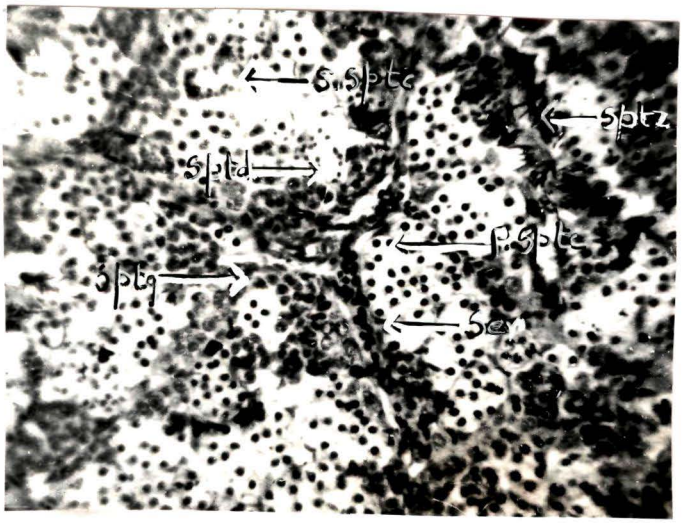
Plate VI Q - A portion of the cross section of the testis of Rana limnocharis Wiegmann during post-breeding period. (x 450)

Plate VI R and S - A portion of the cross section of the testis of Rana limnocharis Wiegmann during different months of the hibernation period. (x 450)

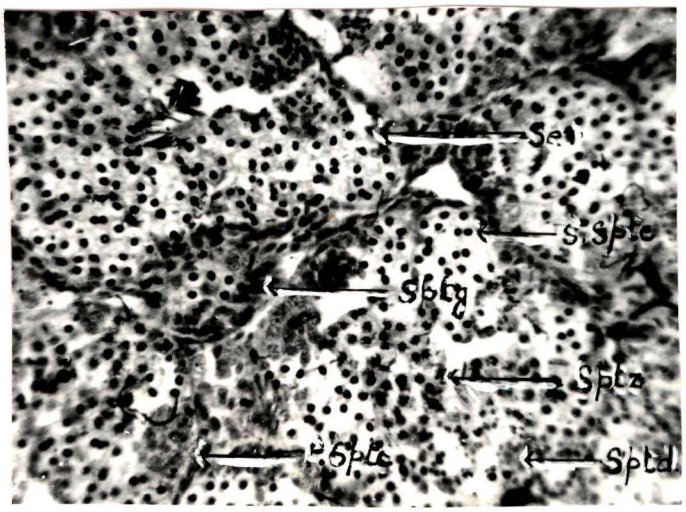
Abbreviations :

Ser	-	Sertoli cell
Sptg	-	Spermatogonia
P.Sptc	-	Primary spermatocyte
S.Sptc	-	Secondary spermatocyte
Sptd	-	Spermatid
Sptz	-	Spermatozoa

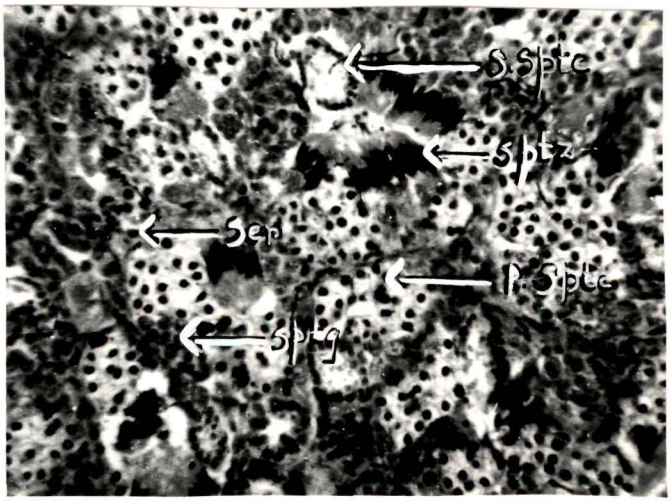
Plate VI



Q



R



S

4. Hibernation Period.

During this period the average number of sections of seminiferous tubules per unit area was 8.98, 11.81, 7.36, 7.56 in the months of November, December, January and February respectively. The following changes were observed in the structures of the tubules. (Fig. 9; Plate IV, I, V, VI R and S).

(a) Sertoli cells : The average number of sertoli cells per unit area was 1.89, 1.98, 2.27, and 2.36; the diameter of the cells was approximately 0.60 mm, 0.66 mm, 0.68 mm and 0.80 mm and their nuclear diameter was approximately 0.07 mm, 0.07 mm, 0.10 mm and 0.26 mm during November, December, January and February respectively.

(b) Spermatogonial cells : The average number of spermatogonial cells per unit area was 14.19, 13.72, 10.69 and 7.85, their diameter was approximately 1.46 mm, 1.04 mm, 1.03 mm and 0.64 mm and their nuclear diameter was approximately 0.13 mm, 0.11 mm, 0.12 mm and 0.10 mm during November, December, January and February respectively.

(c) Primary spermatocytes : The average number of primary spermatocytes per unit area was 16.37, 17.41, 23.94 and 14.86; their approximate diameter was 0.84 mm, 0.87 mm, 0.93 mm and 0.90 mm and their nuclear diameter was approximately 0.09 mm, 0.10 mm, 0.09 mm and 0.10 mm respectively.

(d) Secondary spermatocytes : The average number per unit area for these cells was 13.62, 14.67, 15.52, 16.75, their cellular diameter was approximately 0.54 mm, 0.50 mm, 0.54 mm and 0.60 mm and their nuclear diameter was approximately 0.06 mm, 0.06 mm, 0.07 mm and 0.07 mm during November, December, January and February respectively.

(e) Spermatids : The average number of spermatids per unit area was 4.63, 6.53, 6.62 and 8.51; their diameter was approximately 0.06 mm, 0.08 mm, 0.10 mm and 0.12 mm and their nuclear diameter was approximately 0.04 mm during November, December, January and February.

(f) Spermatozoa : The number of spermatozoa per unit area was observed to be 75, 99, 129 and 203 during November, December, January and February respectively.

The above observations reveal that the number of cross sections of the seminiferous tubules per unit area was maximum during the hibernating period and minimum during the breeding period; their size being largest during breeding period and smallest during hibernation period. The number of sertoli cells was maximum during early breeding period and minimum during post-breeding period; their cellular size being maximum in late hibernating period and minimum during late breeding period. The number of spermatogonial cells was greatest during post-breeding period and lowest during

late breeding period; their cellular size being maximum during early hibernation period and minimum in breeding period. The number of primary spermatocytes was maximum during hibernating period and lowest in the active breeding period; their size was also maximum during hibernation and lowest in the breeding period. The number of secondary spermatocyte was maximum and their size largest during prebreeding and early breeding period; while during late breeding period, their number as well as the size both were minimum. The number of spermatids was maximum during breeding period and minimum in the post and early hibernating months; their cellular size being largest during and early breeding period. The number of spermatozoa was observed to be largest during breeding period and smallest in the early hibernating period.

THE OVARY

(A) MORPHOLOGY OF THE OVARY. (Plate III H)

The ovaries of the frog are paired, multi-lobed organs, attached to the dorsal body wall by a double layered extension of the peritoneum, known as mesorchium. This peritoneum continues around the entire ovary as the theca externa. Each lobe of the ovary is hallow and its cavity is continuous with the other lobes. The size of the ovary varies with the seasons. During early breeding period, the paired ovaries fill the whole of the body cavity and distend the abdomen. The mature eggs are highly pigmented on

the surface of the animal pole, so that the ovary has a speckled appearance of black pigment and white yolk, representing the animal and vegetal poles of the mature egg.

HISTOLOGICAL STRUCTURE. (Fig 10 A,B & C)

As seen in a section, the ovary is surrounded by peritoneal covering the theca externa, from which arise internally a number of individual sacs, each made up of another membrane known as the theca interna or cystwall, in which smooth muscle fibres can be observed. The theca interna surrounds each egg except for the limited area bulging towards the body cavity, where it is covered by only the theca externa. This region ruptures during ovulation to allow the egg to escape from its follicle into the body cavity. The theca externa and the follicle cells together comprises the ovarian follicle. This has the supply of both blood vessels as well as nerves. Within each follicle are found follicle cells, with their oval and granular nuclei, derived orignally from oogonia. These follicle cells surround the developing oocyte and are found in close association with it throughout the process of maturation which occurs within the follicle. Enclosed within each follicle cells, and closely applied to each mature egg, is the non-cellular transparent vitelline membrane, probably derived from both the ovum and follicle cells. This membrane is developed and applied to the egg

Diagrammatic representation of :

Fig. 10 A - Cross section of the growing oocyte of Rana limnocharis Wiegmann.

Fig. 10 B - Cross section of the primary oocyte with pigment of Rana limnocharis Wiegmann.

Fig. 10 C - Cross section of the secondary oocyte of Rana limnocharis Wiegmann.

Abbreviations :

Te	- Theca externa	Nol	- Nucleus
Ti	- THeca interna	Nolo	- Nucleolus
Flc	- Follicle cell	Ap	- Animal pole
Vp	- Vegetal pole	Pgm	- Pigment
Blv	- Blood vessel	Ykg	- Yolk globules
Pfr	- Point of follicular rupture		

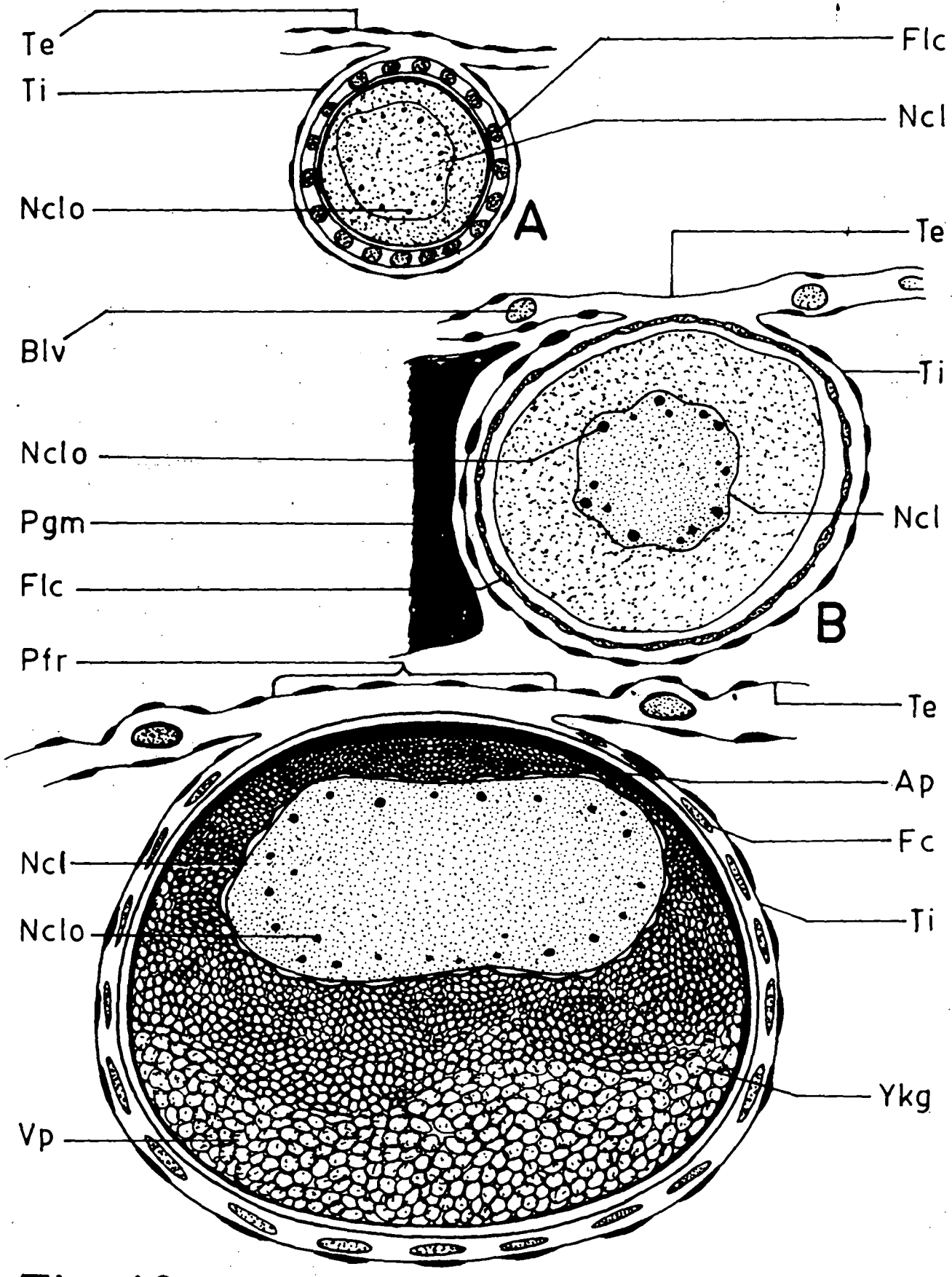

Te
Ti
Nclo
Flc
Ncl
A
Te
Blv
Ti
Nclo
Pgm
Flc
Ncl
B
Pfr
Te
Ap
Fc
Ncl
Nclo
Ti
Ykg
Vp
C

Fig. 10

during the maturation process so that it is not seen around the younger oogonia. As the oocyte enlarges and matures, the follicle cells and membranes are so stretched and flattened that they are not easily distinguished.

The mature egg, as seen in the sections of the ovary is a large sac of yolk, the heavier and larger granules of which are concentrated at the vegetal pole. It has a thin outer layer of cytoplasm, which is more concentrated towards the animal hemisphere and in the region surrounding the entire egg is covered by a pigmented coat which has been described in text books as non-living coat.

(B) CHANGES IN THE OVARY DURING ANNUAL CYCLE.

During early breeding season in April, the ovary was observed full of mostly mature ova. As soon as the spawning started, different developing stages of the ovum were observed. Arising from the germinal epithelium large number of oogonia with or without pigment and devoid of any yolk were observed in the sections of the ovary. Each oogonium was observed to be surrounded with a number of follicle cells. The growing oogonia were distinguished by the accumulation of yolk and the displacement of nucleus to one side, towards the animal pole. The chromatin material becomes achromatic and large number of nucleoli are seen in the nucleus. Nucleus become germinal vesicle with wavy out line. Varying sizes of primary oocyte were seen according

to the amount of yolk accumulated. Secondary oocyte were distinguished as fully grown oocyte with normal nucleus. Maturation of secondary oocyte was not studied but as described by other workers such as Rugh (1951) occurs just at the time of ovulation. Gross changes in the ovary during different periods of annual cycle were observed as described below. (Table V; Plate VII T,U,V,W and Plate VIII X,y & Z)

1. Pre-breeding Period.

The ovaries were observed to contain large number of fully grown oocyte filling the whole of the body cavity.

2. Breeding Period.

During early breeding period the ovaries were observed still occupying the whole of the body cavity. As spawning occured, the size of the ovary was seen to have become smaller; and by the end of the breeding period in August, the ovaries were much reduced in size. Females were spent and histological sections of the ovaries showed very few well developed oocytes. Large number of developing oogonia were observed in the sections of the ovary during April when the spawning starts and in the following active breeding period.

3. Post-breeding Period.

During post-breeding period the sections of the ovaries showed a very large number of primary oocytes in different stages of growth. Mature oocytes were rarely seen.

Plate VII T - A portion of the cross section of the ovary of Rana limnocharis Wiegmann showing growing oocyte. (x 60)

Plate VII U - A portion of the cross section of the ovary of Rana limnocharis Wiegmann showing growing oocyte and pigment cell. (x 60)

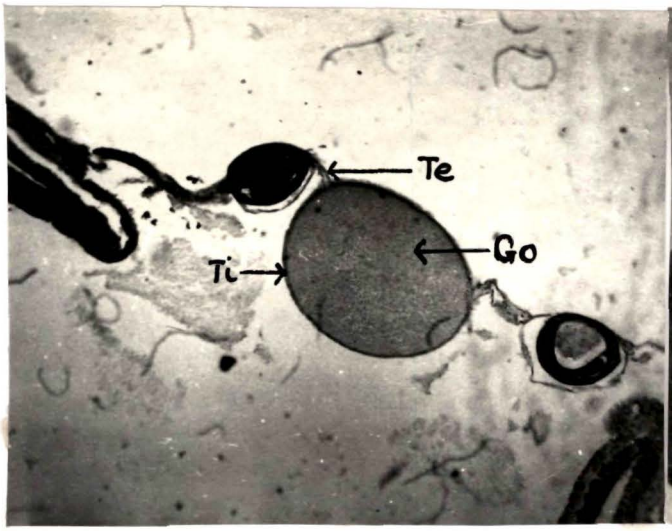
Plate VII V - A portion of the cross section of the ovary of Rana limnocharis Wiegmann showing primary oocyte. (x 60)

Plate VII W - A portion of the cross section of the ovary of Rana limnocharis Wiegmann showing secondary oocyte. (x 60)

Abbreviations :

Te - Theca externa	Nol - Nucleus
Ti - Theca interna	Pgm - Pigment cell
Gdo - Growing oocyte	Nolo- Nucleolus
Po - Primary oocyte	Flc - Follicle cell
So - Secondary oocyte	Ap - Animal pole
Ykg - Yolk globules	Vp - Vegetal pole

Plate VII



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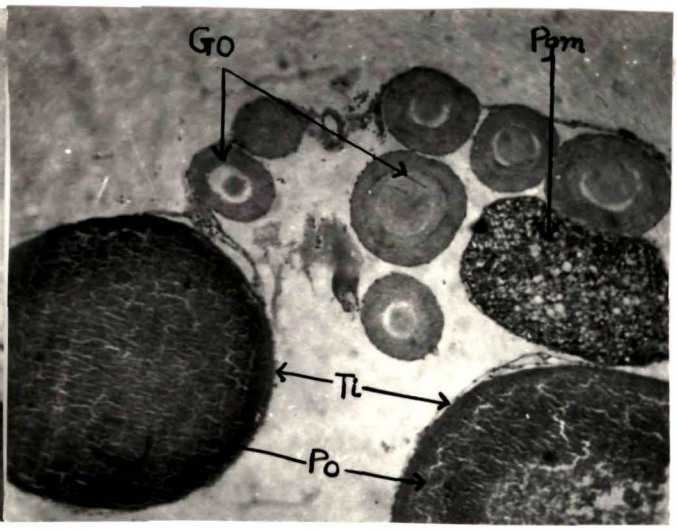
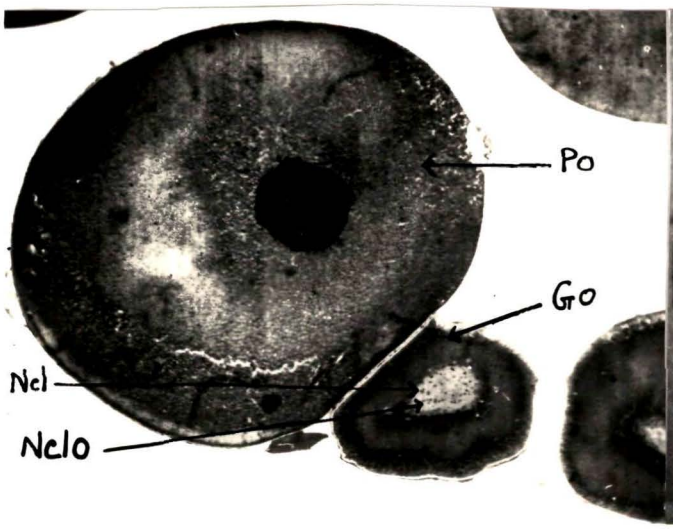
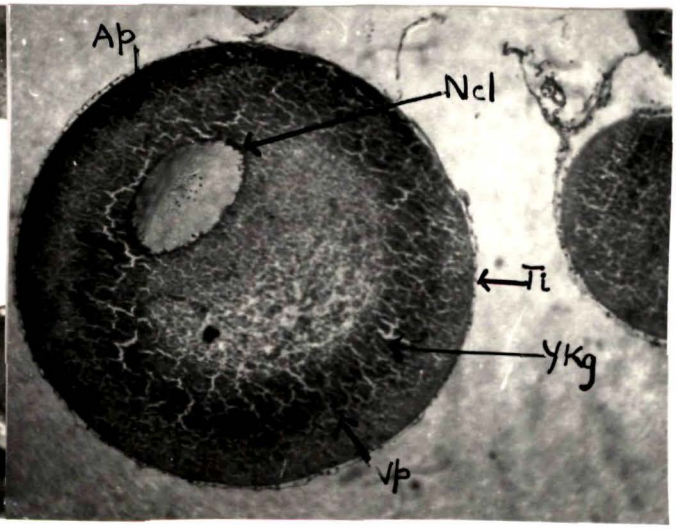


U



V



W

Plate VIII X - A portion of the cross section of the ovary of *Rana limnocharis* Wiegmann showing growing oocyte and peripheral region of mature oocyte. (x 100)

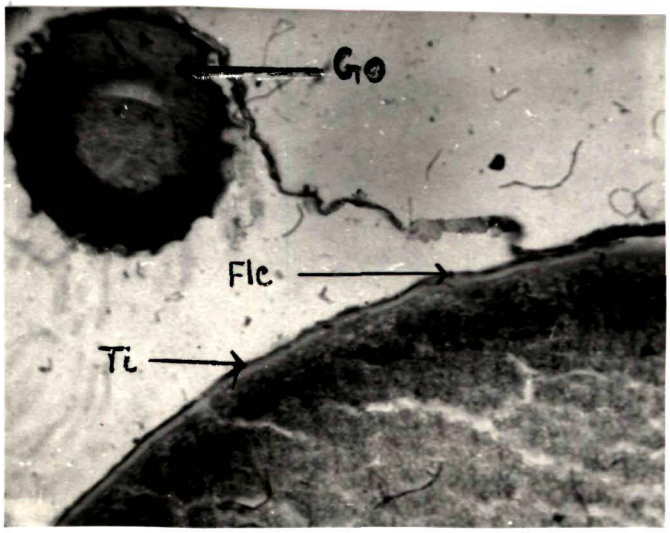
Plate VIII Y - A portion of the cross section of the ovary of *Rana limnocharis* Wiegmann showing pigment cell and primary oocyte. (x 100)

Plate VIII Z - A portion of the cross section of the ovary of *Rana limnocharis* Wiegmann showing secondary oocyte. (x 100)

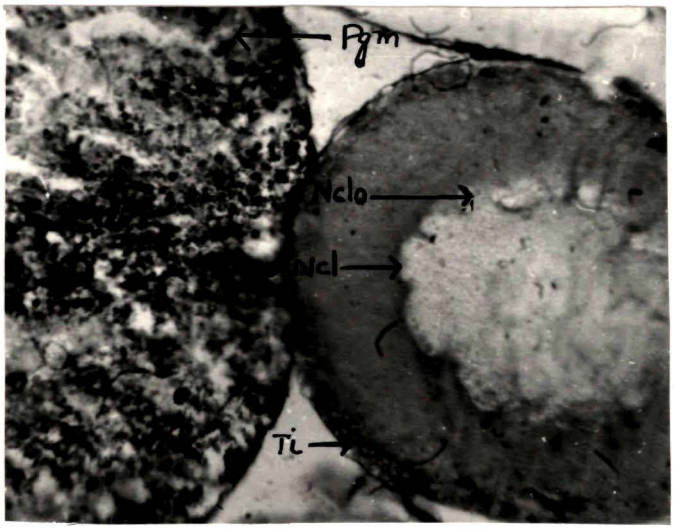
Abbreviations :

Go - Growing oocyte	Ncl - Nucleus
Po - Primary oocyte	Nclo - Nucleolus
So - Secondary oocyte	Flc - Follicle cell
Ti - Theca interna	Pgm - Pigment cell

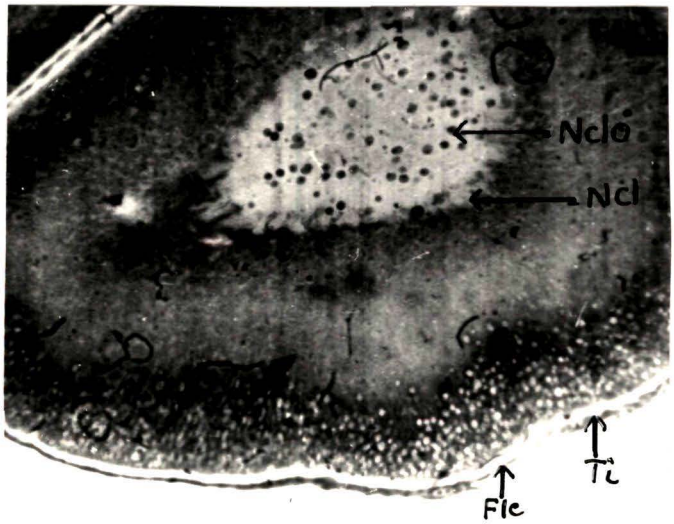
Plate VIII



X



Y



Z

TABLE V

Size of Oocytes in the ovary of Rana limnocharis Wiegmann during different periods of annual cycle.

Period	Month	Size range (Diameter) of Oocytes
Hibernation	February	0.08 - 0.43 mm
Pre-breeding	March	0.08 - 0.93 mm
Breeding	April	0.15 - 0.90 mm
Breeding	May	0.10 - 0.93 mm
Post-breeding	October	0.03 - 0.05 mm

4. Hibernation Period.

During early hibernation period large number of well grown primary oocytes were observed. Dissection of females revealed gradual increase in size of the ovaries during November, December, January and February. Study of the sections of the ovary at the end of the hibernation period revealed the presence of large number of mature oocytes and several oocytes in different maturing stages.

DISCUSSION

The pituitary gland plays a key role in the life processes of the vertebrates. The different cell types of anterior pituitary (pars distalis) secrete various hormones which play an important role in the growth, regulation and maintenance of the vertebrate body. Although the pituitary gland is a most important endocrine gland, there are still controversies regarding the nomenclature and functioning of the different cell types in the pituitary gland. Since 1892, when Schönemann for the first time described two chromophil cell types, eosinophils and cyanophils, several workers tried to distinguish the different cell types by the use of various staining techniques. Not only their nomenclature but also the hormones they secrete is still somewhat controversial especially in Amphibia. In two recent exhaustive reviews, one by Purves (1966) on "The cytology of

the adenohypophysis" and another by Hanke (1976) on "Frog Neuroendocrinology", only three major cell types - the acidophils, basophils and chromophobes have been described as commonly accepted by many workers. And now sub-types of the acidophils and basophils (Kerr, 1965; Mira-Moser, 1970; Van Kemende, 1974; Pehlemann, 1974) have also been described. In the present investigation, behaviour of only three major types of cells has been studied during different periods of the annual cycle of Rana limnocharis.

The histological study of the testis and ovary of Rana limnocharis shows distinct phases of spermatogenesis and oogenesis in relation to the different periods of the annual cycle. The spermatogenic activity was maximum during breeding period from April to August when largest number of spermatozoa ranging from About 308.4 to 424.5 per unit area were observed in each section of the seminiferous tubule in contrast to hibernation period from October to January when their number in each section ranged from 298 to 129 per unit area. It was observed to increase from February (203 per unit area) which is the last month of hibernation. The ovary also showed changes having largest number of mature ova during early breeding season. Their number decreased during active breeding period obviously due to spawning. During late breeding and post-breeding period, active oogenesis was observed. The growth of these oocytes was observed during the hibernation period, so that

the ovary was full of mature ova in February during the last phase of the hibernation period. Detailed histological changes in the ovary could not be studied as it was difficult to get the frogs regularly during this period. The role of pars distalis of the pituitary of Rana limnocharis in controlling these different phases of gametogenesis and breeding activity are clearly correlated with the activity of the three cell types (acidophil cells, basophil cells and chromophobes) found in it.

The acidophil (α) cells charcterized by their spherical to ellipsoidal shape, centric nucleus and acidophilic cytoplasm were observed to be maximum in number (22.19 per unit area) during the egg laying period in June. After the breeding or egg laying period is over, it gradually decreased and became minimum (3.88 per unit area) during the hibernating period in January. Immediately after the period of hibernation, the number started increasing again at the pre-breeding period reaching maximum during the breeding season. The size of the acidophil cells was also maximum (0.16 - 0.15 mm) during the breeding period and minimum at hibernation (0.12 - 0.10 mm). These findings conform those of Zysk (1975), who also describes increase in the number of acidophil cells in breeding period and decrease after the egg laying period up to hibernation. The present findings, however, do not corroborate with the observations of Lakshman (1965) and Rastogi and Chieffi (1970),

who reported smallest number of these cells during the period of egg laying in amphibians. The increase in the number of acidophil cells during late breeding season of Rana limnocharis correlates with its active life and active feeding activity. Juszczyk et al (1966) and Krawczyk (1970) have reported increased weight of intestinal mucosa and high lipid content in the walls of gastrointestinal tract of frogs during such active periods. High level of STH (Somatotrophic hormone) required for synthesis during active gametogenesis. This appears to be a reason why acidophil cells were observed to be in the active phase in Rana limnocharis during later half of the breeding period.

The basophilic (β) cells, identified by their spherical or elongated shape, eccentric nucleus and basophilic cytoplasm, show an increase in their number and high secretory granules in their cytoplasm during the most active phase of the gonads. The number of basophilic cells started as soon as they come out of hibernation in March and became maximum (37.08 per unit area) in July during breeding period. Soon after the breeding period, when egg laying and active phase of spermatogenesis was over, the number started decreasing both in males and females becoming minimum (5.20 per unit area) in the hibernation period. Along with the increase in number, the size of the cells was also observed to be largest during the breeding period, reaching the maximum (0.24 - 0.13 mm) in June and again becoming minimum

(0.14 - 0.13 mm) in December. These observations support Zuber-Vogeli (1953), Orbm (1960, 1961) and Zysk (1975) who reported that the basophilic cells are actively involved in the production of gonadotrophins, such as FSH (Follicle-stimulating hormone) and LH (Luteinizing hormone) controlling the gonadal function as well as the development of secondary sex characters. Increase in the activity of the basophilic cells during breeding season show high level of gonadotrophins. This correlates with the breeding activity including the release of eggs in females and spermatozoa in males. The basophilic delta (δ) cells are supposed to be the source of TSH (Thyreotrophic hormone) and ACTH (Adrenocorticotrophic hormone). As the present investigation was not aimed to find out the relationships of pituitary cells with thyroid activity, the investigation on the basophilic delta (δ) cells was not carried out.

The third type of cells, the chromophobic cells, characterized by a centric nucleus, poorly staining cytoplasm having no definite shape, showed greatest reduction in number (1.98 per unit area), when the number of the basophilic cell was maximum in the breeding period. The size of these cells was observed to be maximum during hibernation (0.22 - 0.16 mm) and minimum during the breeding period (0.10 - 0.08). Similar observations have been made by Zysk (1975). Ackermann, Nowicki and Sarneka-Kellar (1971) suggested these cells to be precursors giving rise to other

cell types. These workers have also reported that their secretion stimulates the thyroid and they also produce ACTH. Rastogi and Chieffi (1970) felt that the chromophobe are degranulated basophil cells, which produce gonadotrophins. The function of chromophobe is thus, still not clear as also reported by Zysk (1975).

On final analysis, it was seen that the three types of cells, 2 chromophil types (acidophils and basophils) and chromophobes in the pars distalis of Rana limnocharis showing distinct phases of activity throughout its annual cycle. The acidophils cells did not show much fluctuation in comparison to the number of basophil cells. The number of acidophil cells increased from pre-breeding to breeding season and started decreasing by the time the frogs started undergoing hibernation. The number of the basophil cells was always found to be more than acidophil cells except during post-breeding period. They were almost double in number than the acidophil cells during pre-breeding period; and about $1\frac{1}{2}$ times more than acidophil cells during breeding season. The cyclic behaviour of the acidophils, basophils and chromophobes correlates well with the Pre-breeding, Breeding, Post-breeding and Hibernation periods of the annual cycle of the animal.

SUMMARY

The changes in the three main types of cells of the pars distalis of the pituitary, viz., acidophils, basophils and chromophobes and the corresponding histomorphological changes in the testis and ovary of Rana limnocharis Wiegmann during different periods of the annual cycle have been investigated. The number and size of acidophil cells increased during pre-breeding and breeding periods and decreased when the frogs entered hibernation. The basophil cells were always more in number than the acidophil cells except during the post-breeding period. Their size was also larger during the pre-breeding period. The size of the basophil cells was larger and their number was almost double than that of the acidophil cells and it reduced to $1\frac{1}{2}$ times during the breeding period. The number of chromophobe cells was maximum during the hibernation period and minimum during the breeding period. These cyclic changes in the cells of the pars distalis were distinctly correlated with the cyclic changes in the gonads. The spermatogenic activity in the testis was maximum during breeding period from April to August when the largest number of spermatozoa were observed in the seminiferous tubules. The ovary contained the largest number of mature ova during early breeding period although this number decreased during the breeding period due to spawning. Active oogenesis was observed during the late breeding and post-breeding periods. Large

number of the basophilic cells (gonadotrophic) during pre-breeding and breeding periods indicates high level of gonadotrophic hormone and correlates well with the onset and progress of breeding activity; and large number of acidophilic cells (somatotrophic) during the late breeding period and post-breeding period indicates high level of somatotrophic hormones and correlates well with high gametogenetic activity.

REFERENCES

Ackermann, J., Z. Nowicki and M. Sarnecka-Kellar. 1971. Cytologia i histologia. PZWL. Warszawa: 283-296.

Disbrey, B.D. and J.H. Rack. 1970. Histological laboratory Methods. E. and S. Livingstone, Edinburgh.

Doerr-Schott, J. 1974. Cyto- immunochemical study of the hypophysical cells of Amphibians by light and electron microscopy. Fortschritte der Zoologie. 22: 245-267.

Emmel, V.M. and E.V. Cowdry. 1970. Laboratory Technique in Biology and Medicine. Robert E Krieger Publishing Co. Inc.

Hanke, W. 1976. 'Neuroendocrinology'. In. Frog Neurobiology. (Edited by R. Llinas and W. Precht): 975-1020. Springer-Verlag Berlin, Heidelberg, New York.

Harris, G.W. and B.T. Donovan. 1966. The Pituitary Gland. Vol. I. Anterior Pituitary. Butterworths. London.

*Herlant, M. 1960. Etude critique de deux techniques nouvelles destinees a mettre en evidence les differentes categories callulaires presentes dans la glande pituitaire. Bull. Micr. Appl. 10: 37-44.

Juszczyk, W., K. Obrsut and W. Zamachowski. 1966. Morphological changes in the alimentary canal of the common frog (Rana temporaria L.) in the annual cycle. Acta biol. cracov. Zool. 9: 239-246.

Kerr, T. 1965. Histology of the distal lobe of the pituitary of Xenopus laevis. Daud. Gen. comp. Endocr. 5: 232-240.

Krawczyk, S. 1970. Changes in the lipid and water content in some organs of the common frog (Rana temporaria L.) in the annual cycle. Acta biol. cracov. Zool. 14: 211-237.

Lach, H. 1970. The relevance of biorythm in detecting and diagnosing illness. Rocz. nauk-dydakt. WSP w KraKowie. Prace Zool. 37: 91-95.

Lakshman, A.B. 1965. Structural changes in the pituitary glands of female frogs and toads during different seasons of the year. Acta. anat. 61: 108-126.

Mallory, F.B. 1900. A contribution to staining methods. J. exp. Med. 5: 15-20.

McManus, J.F.A. 1946. Histological demonstration of mucin after periodic acid. Nature. Lond. 158: 202.

Mira-Moser, F. 1970. L'ultrastructure de l'adenohypophyse du crapaud Bufo bufo L. I. Identification des types cellulaires et comparaisons des resultats obtenus avec deux fixateurs differents. Z. Zellforsch. 105: 65-90.

Ortman, R. 1956. Two types of acidophils in the anterior pituitary of the frog and their relation to the periodic acid-Schiff reaction. J. Histochem. 4: 471-475.

Ortman, R. 1960. The periodic acid-Schiff positive cells of the frog pars distalis : their tinctorial transmutation. Anat. Rec. 137: 386.

Ortman, R. 1961. Anterior lobe of pituitary of Rana pipiens. A cytological and cytochemical study. Gen. comp. Endocr. 1: 306-316.

Pearse, A.G.E. 1953. Cytological and cytochemical investigations on the foetal and adult hypophysis in various physiological and pathological states. J. Path. Bact. 65: 355-370.

*Pehlemann, F.W. 1974. Funktionsmorphologie der Adenohypophyse von Anuren. In: Vergleichende Endokrinologie (W. Hanke and M. Lindauer, eds.) Fortschritte der Zoologie. 22: 204-227. Stuggart : G. Fischer.

Purves, H.D. 1961. Morphology of the hypophysis related to its function. In: Sex and Internal secretions. 3rd edn. Chapter 3. Ed. W.C. Young, Williams and Wilkins. Baltimore.

Purves, H.D. 1966. 'Cytology of the adenohypophysis'. In: The Pituitary Gland. Vol. I. Anterior Pituitary. 147-232: Butterworths. London.

Rastogi, R.K. and Chieffi, G. 1970. Cytological changes in the pars distalis of pituitary of the green frog, Rana esculenta L., during reproductive cycle. Z. Zellforsch. 111: 505-518.

Reyrel, R. 1967. Cytological observations on the anterior hypophysis of Pelobates cultrives (Amphibia: Anura). C R Hebd. Seances. Acad. Sci. Ser. D Sci. Natur. (Paris) 265(9): 695-697.

*Romeis, B. 1940. Die Hypophyse. In Handbuch der Mikroskopischen Anatomie des Menschen. Vol. 6 Part 3. Ed. von Mollendorff. Julius Springer. Berlin.

Rugh, R. 1951. The Frog. Its reproduction and development. McGraw-Hill Book Company. Inc.

*Schonemann, A. 1892. Hypophysis und Thyreoida. Arch. path. Anat. 129: 310-336.

Van Kemenade, J.A.M. 1974. Regulation in the interrenal gland and demonstration of cell types in the pars distalis

of amphibia. In: Vergleichende Endokrinologie (W. Hanke and M. Lindauer, eds.) Fortschritte der Zoologie 22: 228-244. Stuggart : G. Fischer.

Van Oordt, P.G.W.J. 1961. The gonadotropin-producing and other cell types in the distal lobe of the pituitary of the common frog Rana temporaria. Gen. comp. Endocr. 1: 364-374.

Van Oordt, P.G.W.J. 1963. Cell types in the pars distalis of the amphibian pituitary. In Cytologie de l'Adenohypophyse. Editions du C.N.R.S. Paris.

Van Oordt, P.G.W.J. 1965. Cyclical changes in the gonadotropin-producing cells of the pituitary and their implication for the regulation of the sexual cycle in the male common frog, Rana temporaria. Arch. Anat. micr. Morph. Exp. 54: 630-631.

*Zuber-Vogeli, M. 1953. L'histophysiologie de l'hypophyse de Bufo vulgaris L. Arch. anat. histol. embryol. 35: 77-180.

Zysk, A. 1975. Quantitative changes in cells of the pars distalis of the pituitary in Rana temporaria (L). during the annual cycle. Acta Biologica Cracoviensia. Zoologia. XVIII: 9-20.

* Not consulted in original.

Chapter 5

Induced - Breeding

INTRODUCTION

First pioneering experiments demonstrating the induction of ovulation in anurans by injecting or implanting homoplastic pituitary glands were performed by Wolf (1929) on Rana pipiens, and Houssay et al (1929) on Bufo marinus. Since then many subsequent Developmental Biologists such as Adams (1931), Wills et al (1933), Rugh (1934, 1935a, 1935b, 1939 and 1962), Creaser and Gorbman (1935), Adams and Granger (1938), Ramaswami and Lakshman (1958 and 1959), Wright (1945 and 1950), Wright and Hisaw (1946), Wright and Flathers (1961), Nieuwkoop and Faber (1967) and Hock and Wen (1970) have confirmed the induction of ovulation in anurans by the injection pf pituitary hormones. They have described different techniques which have become laboratory procedures in many countries. In United States of America certain companies* supply pituitary kits for demonstrating of induced breeding for classroom experiments. Most Indian laboratories, however, still depend upon the frog spawns collected from the natural resources for developmental studies. In 1959, Ramaswami and Lakshman observed, "in most Indian Universities demonstration

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* e.g. 1. Carolina Biological Supply Company, Burlington, N.C. 27215.

2. Turtox, General Biological Supply Co. Inc. 8200, Hoyne Avenue, Chicago ILL-60620, U.S.A.

of frog embryology has not been undertaken as the technique of spawning and fertilizing the eggs under laboratory conditions to follow up their further fate is not adequately developed". They also outlined a technique for induced breeding in Rana cyanophlyctis, but the situation has not much changed. Use of the fresh homoplastic pituitaries or mammalian hormones or a combination of the two has been prescribed by different workers in different anurans. It has been experienced that the females of certain common frog species found in India, for example, Rana tigrina, Rana hexadactyla do not respond to mammalian gonadotrophins available in local market. Rana cyanophlyctis has, however, responded to androgens, progesterone and deoxycorticosterone acetate (Ramaswami and Lakshman, 1959). Collection and injection of the fresh frog pituitaries has hot been found convenient at every place due to inadequate supply of mature frogs or other technical reasons.

The technique of induced breeding evolved during the course of the present investigation on Rana limnocharis Wiegmann can be conveniently and successfully followed in any laboratory. It is a modification of Rugh's (1934) technique based on the techniques of Osche (1948) and Jhingran (1975). According to this technique a stock of the anterior pituitaries preserved in absolute ethyl alcohol has to be built up; and at the time of experiment injection of the homogenate of these pituitaries is given

to the females in prescribed dosage. Eggs are normally obtained by stripping on the next day. They are immediately fertilized; and culture of developing embryos is maintained. This technique has been found to yield more successful results than other techniques.

REVIEW OF LITERATURE

In 1929, Wolf studied the effect of transplantation of pituitary on reproduction of Rana pipiens. He removed its anterior lobe of pituitary and inserted it into the lateral or femoral lymph sinus of another female receipient and observed that, it induced sexual maturity and ovulation in the female. Houssay, Guisti and Lascano-Gonzalez (1929) confirmed it with similar results in Bufo marinus. Wills, Riley and Stubbs (1933) showed ovulation in toads can be induced by fish pituitary. Rugh (1934 and 1935a) performed similar experiments with homoplastic pituitaries on various species of frogs and toads found in North America. He showed that it induces amplexus and ovulation and such eggs can be artificially inseminated. In 1935a, he prescribed dosage for inducing ovulation and method to secure fertilized eggs. He demonstrated that extracts of the mammalian anterior pituitary (sheep gland or antuitrin - S from pregnancy urine) are effective in toads but not uniformly effective in frogs. In his another publication during the same year (1935b) he prescribed

injection of anterior pituitary in distilled water or 10% ethyl alcohol and concluded that the degree to which the ovaries are emptied, depends upon the dose of the hormone injected and the time of ovulation depends upon temperature. He investigated the breeding behaviour of different frogs found in North America and stated that anuran eggs and larvae can be obtained in all months. Adams and Granger (1938) showed that Triturus viridescens pituitaries induce ovulation in Rana pipiens and felt that there was no zoological specificity of gonad stimulating hormone. In 1939, Rugh described in detail the technique for obtaining the anterior pituitary gland and demonstrated that Rana pipiens collected from hibernation can be induced to ovulate by anterior pituitary injection much before its actual breeding period. In 1941, Landgrebe and Purser described the technique of breeding Xenopus in the laboratory. In 1942, Robinson and Hill studied the induced ovulation in Rana pipiens. In the same year, Creaser demonstrated the induction of ovulation in Rana pipiens by bird pituitary preparations. In 1945, Wright demonstrated in vitro ovulation from the ovaries by placing them in the culture medium to which anterior pituitary hormmne was already added. In 1950, he showed that length of exposure of ovariesmto the hormone solution was responsible for "in vitro" ovulation. In 1946, Wright and Hisaw studied the effect of mammalian pituitaries on the ovulation of

Rana pipiens. In 1961, Wright and Flathers showed that although Rana pipiens were insensitive to ovulatory influences, injection of frog pituitary gland in association with progesterone induces complete ovulation in it. Barr and Hobson (1967) formulated the method of estimating the number of eggs laid by Xenopus laevis by injecting gonadotrophin. While studying the experimental ovulation and fertilization of Rana ridibunda, Alonso-Bedate and Serrano (1970) described that, keeping females at room temperature detoriates the ovaries. During the same year Hock and Wen induced artificial breeding in Rana limnocharis Boie according to the Rugh's (1935) method and traced its early development up to tadpole stage.

Among the frog species available in India, the induced breeding has been attempted by Ramaswami and Lakshman (1959) on Rana tigrina, Rana cyanophlyctis and Rana hexadactyla. They reported that many mammalian hormones, such as, follicle-stimulating hormone (FSH), luteinizing hormone (LH), pregnant mare serum, chorionic gonadotrophin, estrogen, adrenocorticotrophic hormone (ACTH), cortisone, somatotrophic hormone (STH), thyroid stimulating hormone (TSH), stimulate ovulation in these frogs only in combination with a threshold pituitary gland dose. They reported that Rana cyanophlyctis can be induced to breed almost throughout the year. They further

demonstrated that Heteropneustes pituitary glands also induces ovulation in Rana cyanophlyctis.

During the last two decades interesting advances have been made in the techniques of induced breeding in fishes by Alikunhi et al (1960) and Choudhuri (1960 and 1963) (for review see Jhingran, 1975). These techniques include preservation of fish pituitary in absolute ethyl alcohol and determining the dosages of pituitary in terms of per gram of the body weight of the female to be injected. This technique is being successfully followed by fish farmers throughout India and many other countries.

MATERIALS AND METHODS

TECHNIQUE OF INDUCED BREEDING.

Collection of the frogs.

For induced breeding alive specimens of Rana limnocharis were collected from the Pologround, Shillong from July to middle of October in 1975 and from late March to middle of October in 1976 and 1977 during their active breeding period. The experiments were repeated in 1978 also. Soon after collection they were removed to the laboratory and maintained in glass aquaria (75.5 cm X 45.5 cm X 45.5 cm) and covered with 1 inch sq. wire mesh coverning. Each aquarium was filled 1/6 with water and

set with stones, water plants and steep sand base on one side of the aquarium to form a sort of natural environment for the frogs.

Collection of the anterior pituitaries.

Anterior pituitary glands were dissected out from the mature male and female frogs according to the method prescribed by Rugh (1934 and 1962) and preserved in absolute ethyl alcohol. Each donor was pithed and decapitated by sharp scissors just posterior to the angle of the jaws. The head was dissected upside down and the anterior pituitary gland was exposed through the roof of the buccal cavity. The parasphenoid was cut along its two sides with the tips of sharp scissors inserted posterior to the position of the gland through the foramen magnum and deflected forward to expose the ventral region of the brain. The anterior pituitary can be easily located as a cream coloured bean shaped structure situated transversely posterior to the optic chiasma and hypophysis. (Figs. See Chapter IV). In the breeding season it is much enlarged. It was removed with forceps and transferred to absolute ethyl alcohol. Acetone preserved pituitaries did not induce ovulation. Vials containing preserved pituitaries in the absolute ethyl alcohol were corked, sealed with wax and kept in the refrigerator. (See Experiments and Results).

Injection technique.

Corning glasswares (beakers, petridishes, watch glasses, specimen tubes) and surgical steel instruments were properly sterilized in autoclave or pressure cooker at a pressure of 15 lbs. for 5 minutes. Pressure cooker was found convenient for sterilization. Mature females frogs were collected from storage aquaria for induction of ovulation. The maturity of the frogs was tested by gently pressing the abdomen and feeling the presence of ova specially in its lower pelvic region. In Rana limnocharis mature ova can be observed in the pelvic region through the translucent integument at the region of groin. In some pilot experiments receipient female was weighed and dosage of pituitaries per gram of the body weight of the female was calculated. The preserved pituitaries were then taken out from the alcohol and weighed on a Keroy's electric chemical balance as per the calculation.

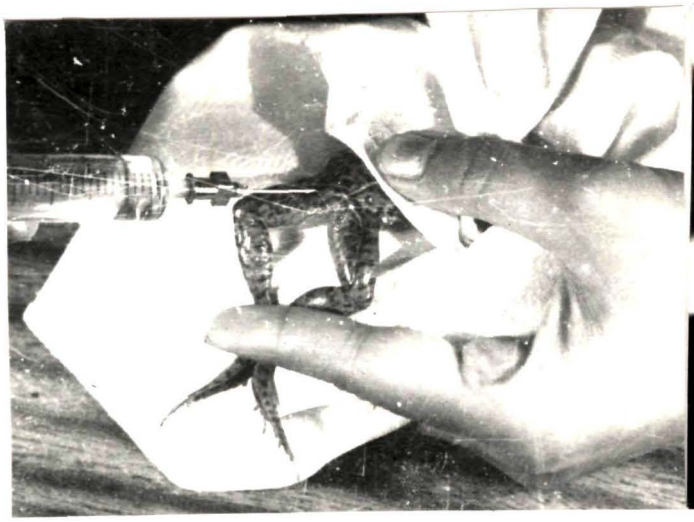
The weighed pituitaries were rinsed by a dip in the sterile water (glass distilled water was boiled in flask and sealed by aluminium foil) in a watch glass and immediately transferred to another watch glass containing 0.5 ml of sterile water and homogenated. Keeping pituitaries for a longer time in water, either at the time of excising them from the donor frogs before preservation absolute ethyl alcohol or at the time of rinsing,

Plate I A - Injection technique.

Plate I B - Stripping of eggs from the injected female.

Plate I C and D - Stripping of eggs in the sperm suspension for fertilization.

Plate I



A

B

C

D

found to reduce their potency. The host female was now held in the left hand and the homogenate was injected through No. 21 injection needle in its dorsal femoral lymph sac by the right hand (Plate IA) as per the technique of Osche (1968). The injected female was kept in a clear jar. Contact of the injected frog with water was avoided as it may cause swelling of jelly of the eggs in cloaca which prevents fertilization.

Stripping of eggs.

The eggs from injected female were obtained by stripping on the next day (Plate IB). In case the experiment was not successful and eggs were not obtained the female was again injected with another dose and the stripping was again attempted on the third day. After experiment the females were released in the pond.

Fertilization of eggs and maintenance of culture.

At the time of stripping as soon as some eggs were seen coming out of the cloaca of the injected female; it was immediately kept back in the jar and a sperm suspension was quickly prepared by macerating 2 testis of a mature male frog in 100 ml of 10% Holtfreter's solution. A drop of sperm suspension was examined on a slide and under a microscope to test the viability of the spermatozoa. Normally it takes 4-5 minutes for a spermatozoa to become active in this culture medium. As soon as active

spermatozoa were observed the eggs from the injected female were stripped directly in the sperm suspension (Plate I C and D). Initiation of the 1st cleavage confirmed that the eggs were fertilized. Sometimes it is better to inject the male frog to ensure getting active sperms at the time of the experiment. Soon after cleavage, the culture medium was changed with a view to rinse the spawn. After hatching the culture medium was replaced by pond water.

The experimental data were carefully recorded and photographs were taken wherever needed.

EXPERIMENTS AND RESULTS

Four types of experiments were performed on the inudction of ovulation in Rana limnocharis from 1975 to 1978. (1) With fresh homoplastic pituitaries. (2) With heteroplastic pituitaries from Bufo melanostictus preserved in absolute ethyl alcohol. (3) With homoplastic pituitaries preserved in absolute ethyl alcohol. (4) With mammalian hormones, such as goat pituitary, sheep pituitary powder, human chorionic gonadotrophins (Physex Leo), antuitrin-S (Parke, Davis and Company).

1. EXPERIMENTS PERFORMED WITH FRESH HOMOPLASTIC PITUITARIES.

Experiments with fresh homoplastic pituitaries were performed in 1975. In some pilot experiments it was

felt that four female pituitaries may induce ovulation. As such, the first experiment was performed on July 18, 1975. Four pituitaries were dissected out from mature females and injected into the abdomen of the female with 0.0 ml distilled water as per technique devised by Rugh (1962). Next day the female laid egg by itself. In the 2nd, 3rd, 4th, 5th, 6th and 7th experiments, performed similarly, no eggs could be obtained by stripping on the next day as well as the third day. Data has been compiled in Table I. On dissection of these injected females, very few mature ova could be observed in the ovary. It was felt that, the dosage of pituitaries injected may not have been sufficient to release the ova.

The eggs obtained in the first experiment were fertilized by releasing them in the sperm suspension and culture of the developing embryos was maintained for further studies.

2. EXPERIMENTS PERFORMED WITH HETEROPLASTIC PITUITARIES DISSECTED OUT FROM BUFO MELANOSTICTUS PRESERVED IN ABSOLUTE ETHYL ALCOHOL.

Jhingran (1975) described the induced breeding in fishes with pituitaries preserved in absolute ethyl alcohol. Following this technique some pilot experiments were performed in 1976. It was felt that alcohol preserved pituitaries yield better results in comparison to

TABLE I

Experiments on Induced breeding in Rana limnocharis with fresh homoplastic pituitaries.

Date	Experiment Number.	Room Temperature.	Numberofbf pituitaries injected.	Amount of distilled water injectęd.	Time of injection (Hours)	Result of stripping	
						after 24 Hours	after 48 Hours
July 18, 1975	75-1	20.3°C	4 (♀)	1.0	14.00	+	
August 6, 1975	75-2	19.4°C	5 (♂)	1.0	15.00	-	-
August 8, 1975	75-3	19.8°C	4 (♂)	1.0	12.00	-	-
August 12, 1975	75-4	18.5°C	4 (♀)	1.00	12.00	-	-
August 14, 1975	75-5	19.0°C	3 (♂) 1 (♀)	1.00	12.00	-	-
September 13, 1975	75-6	17.1°C	5 (♂)	1.0	12.00	-	-
September 17, 1975	75-7	17.0°C	3 (♂) 1 (♀)	1.0	12.00	-	-

+ obtained the eggs

- no egg could be obtained.

the fresh pituitaries. Acetone preserved pituitaries did not induce ovulation in Rana limnocharis.

Bufo melanostictus were obtained from Gauhati as they were available in large numbers there at the time of experimentation in 1976. Experiments were performed on Rana limnocharis soon after they came out from hibernation in March/April at Shillong. The condition of the female was properly examined and only these females which had mature ova, as detected by a 'feel' by gently pressing the abdomen, were selected for experimentation. The mature ova could also be seen in many cases in the region of groin through the translucent integument. The pituitaries were dissected out from Bufo melanostictus and preserved in absolute ethyl alcohol. The data has been presented in Table II. In the first four experiments (76-1, 76-2, 76-3, 76-4) a higher dosage i.e. the six preserved pituitaries were used and in the last two experiments (76-5, 76-6) five and four preserved pituitaries were used respectively. The females to be injected were weighed. The pituitaries were taken out from the absolute alcohol, weighed, homogenised in 0.5 ml glass distilled water and injected in the dorsal lymph sac of the females as per the technique followed in the South African toad, Xenopus laevis (Nieuwkoop and Faber, 1967). The stripping of eggs was attempted on the next day in each experiment. It was observed that the females weighing

TABLE II

Experiments on Induced breeding in Rana limnocharis with pituitaries of Bufo melanostictus preserved in absolute ethyl alcohol.

Date	Experiment Number	Room Temperature	Weight of injected female (gm)	Number of pituitaries injected	Amount of distilled water injected (ml)	Total weight of pituitaries (mg)	Weight of pituitaries mg per gm of the body weight of the receipient.	Time of injection (Hours)	Time of stripping next day (Hours)	Number of eggs stripped
April 26, 1976	76-1	22.0°C	15.2	6 (♀)	0.5	1.8	0.12	12.30	13.00	178
"	76-2	22.0°C	12.1	6 (♀)	0.5	1.9	0.15	13.00	13.10	189
"	76-3	22.0°C	12.5	6 (♀)	0.5	1.8	0.15	13.15	13.20	182
May 5, 1976	76-4	23.0°C	15.7	6 (♂)	0.5	1.6	0.10	13.00	13.45	341
"	76-5	23.0°C	12.1	5 (♂)	0.5	0.8	0.06	13.20	14.00	Nil
"	76-6	23.0°C	10.2	4 (♂)	0.5	0.5	0.05	13.40	14.10	Nil

above 12 gms responded to the pituitary injection and a dosage of Bufo melanostictus pituitaries above 0.10 mg per gm of the body weight of the females induced them to release the eggs. Thus experiment Nos. 76-1, 76-2, 76-3, 76-4 were successful. In experiments Nos. 76-5 and 76-6 the dosage appeared to be lone and so the females were not induced to release the eggs.

Eggs were fertilized by stripping them in the sperm suspension in each experiment and then culture was maintained.

3. EXPERIMENTS WITH HOMOPLASTIC PITUITARIES OF RANA LIMNOCHARIS PRESERVED IN ABSOLUTE ETHYL ALCOHOL.

Series 1. (With freshly preserved pituitaries).

As Rana limnocharis was available in plenty at Shillong in April and onwards, the 1st Series of these experiments were performed with their homoplastic pituitaries. Keeping in mind the results of experimentation with heteroplastic pituitaries of Bufo melanostictus, the dosage of alcohol preserved pituitaries were kept higher than 0.10 mg per gm of the body weight of the females in four out of eight experiments and lower than it in the other four experiments. The data has been tabulated in Table III. Experiments 76-7, 76-8, 76-9, 76-14 were successful. Experiments 76-10, 76-11, 76-12, 76-13 did not yield successful results. These results

TABLE III

Experiments on Induced breeding in Rana limnocharis with homoplastic pituitaries preserved freshly in absolute ethyl alcohol.

Date	Experiment Number	Room Temperature	Weight of injected female (gm)	Number of pituitaries injected	Amount of distilled water injected (ml)	Total weight of pituitaries (mg)	Weight of pituitaries mg per gm of the body weight of the receipient.	Time of injection (Hours)	Time of stripping next day (Hours)	Number of eggs stripped.
May 5, 1976	76-7	23.1°C	12.1	11 (♂)	0.5	1.4	0.11	13.15	11.45	265
May 11, 1976	76-8	20.0°C	12.8	6 (♀)	0.5	1.2	0.10	13.45	11.30	289
June 16,1976	76-9	19.2°C	10.9	6 (♀)	0.5	1.2	0.12	11.45	11.30	167
June 30,1976	76-10	20.3°C	14.0	4 (♀)	0.5	1.1	0.07	11.45	11.30	Nil
"	76-11	20.3°C	10.1	3 (♀)	0.5	0.6	0.06	11.30	11.45	Nil
July 1, 1976	76-12	20.0°C	14.2	6 (♂)	0.5	1.0	0.07	12.10	12.20	Nil
July 3, 1976	76-13	10.2°C	12.4	6 (♂)	0.5	0.8	0.06	17.30	16.00	Nil
July 12,1976	76-14	18.1°C	14.9	5 (♀)	0.5	1.7	0.12	15.35	Eggs laid by itself	

indicate that during May, June and July a dosage of the homoplastic pituitaries above 0.10 mg per gm of the body weight of the females caused induction of ovulation in the females without any difficulty.

Series 2. (With pituitaries preserved soon after the period of hibernation).

The 2nd Series of experiments were performed soon after the period of hibernation in 1977 with homoplastic pituitaries of Rana limnocharis preserved freshly in absolute ethyl alcohol after hibernation period. A dosage of the pituitaries per gram of the body weight of females lower than that determined in the 1st and 2nd Series of experiments was attempted, as the level of gonadotrophic hormone was expected to be higher in the females soon after hibernation was over. The data has been compiled in Table IV. Experiments 77-1, 77-2, 77-3 were not successful. The experiments 77-4, 77-5, 77-6 were successful. The results indicate that dosages of the pituitaries 0.06, 0.07, 0.08 mg per gm of the body weight of the females in experiment Nos. 77-4, 77-5, 77-6 induced ovulation in the females weighing above 16 gms.

Eggs obtained were stripped into sperm suspension, fertilized and allowed to develop in each experiment for further study.

TABLE IV

Experiments on Induced breeding in Rana limnocharis with homoplastic pituitaries preserved in absolute ethyl alcohol soon after the hibernation period.

Date	Experiment Number	Room Temperature	Weight of injected female (gm)	Number of pituitaries injected.	Amount of distilled water injected. (ml)	Total weight of pituitaries. (mg)	Weight of pituitaries mg per gm of the body weight of the receipient.	Time of injection. (Hours)	Time of stripping next day. (Hours)	Number of eggs stripped.
March 30,1977	77-1	23.5°C	15.2	4 (♀)	1.0	0.8	0.05	13.10	14.15	Nil
"	77-2	23.5°C	12.4	5 (♀)	1.0	0.92	0.07	14.50	15.20	Nil
April 19,1977	77-3	20.0°C	13.0	6 (♀)	1.0	1.0	0.07	15.15	15.00	Nil
May 3, 1977	77-4	22.5°C	17.0	5 (♀)	1.0	1.1	0.06	12.00	11.45	590
May 10, 1977	77-5	25.8°C	16.5	5 (♀)	1.0	1.2	0.07	12.00	11.00	840
"	77-6	25.8°C	17.0	6 (♀)	1.0	1.4	0.08	12.20	11.30	780

Series 3. (With pituitaries preserved for one year).

The 3rd Series of experiments were performed during the second half of April, 1977 and onwards with homoplastic pituitaries of Rana limnocharis preserved in absolute ethyl alcohol for 1 year. It was observed that the potency of the pituitaries was retained for 1 year and the homoplastic pituitaries so preserved acted as effectively as the freshly preserved pituitaries. The data has been compiled in Table V. Experiments 77-A, 77-B, 77-C, 77-E, 77-F, 77-G, 77-H were successful and experiments 77-D, 77-I were not successful. In experiment 77-D the dosage of pituitaries 0.05 mg per gm of the body weight was two lone and in experiment 77-I the experimented female, when dissected, was found to contain very few mature ova.

Eggs obtained were stripped into sperm suspension, fertilized and allowed to develop.

Series 4. (With pituitaries preserved for two years).

The 4th Series of experiments were performed in 1977 with homoplastic pituitaries of Rana limnocharis preserved in absolute ethyl alcohol for 2 years. In this Series also, as in Series 3, the potency was seen to have been retained for 2 years and acted as effectively as freshly preserved pituitaries. The data has been compiled

TABLE V

Experiments on Induced breeding in Rana limnocharis with homoplastic pituitaries preserved in absolute ethyl alcohol for one year.

Date	Experiment Number	Room Temperature	Weight of injected female (gm)	Number of pituitaries injected.	Amount of distilled water injected. (ml)	Total weight of pituitaries. (mg)	Weight of pituitaries mg per gm of the body weight of the receipient	Time of injection. (Hours)	Time of stripping next day. (Hours)	Number of eggs stripped.
April 13,1977	77-A	16.0°C	16.00	6 (♂)	0.5	1.4	0.08	12.45	9.30	472
April 14,1977	77-B	17.0°C	14.30	6 (♂)	0.5	1.2	0.08	11.55	10.30	1462
"	77-C	17.0°C	12.00	7 (♂)	0.5	1.0	0.08	12.05	11.30	110
April 19,1977	77-D	20.0°C	15.40	5 (♂)	0.5	0.8	0.05	11.55	12.00	Nil
"	77-E	20.0°C	17.00	6 (♂)	0.5	1.2	0.07	12.25	14.00	1362
"	77-F	20.0°C	15.00	7 (♂)	0.5	1.1	0.07	14.45	14.10	840
April 26,1977	77-G	23.5°C	20.50	6 (♂)	0.5	1.4	0.06	11.55	11.15	1457
"	77-H	23.59C	16.00	7 (♂)	0.5	1.6	0.1	12.15	11.35	973
"	77-I	23.5°C	13.20	8 (♂)	0.5	1.1	0.08	12.45	12.00	Nil

in Table VI. Five experiments out of seven, viz, 77-b, 77-c, 77-f, 77-g, 77-h were successful. In each experiment eggs obtained were stripped into sperm solution, fertilized and allowed to develop.

Series 5. (With 2 year old or freshly preserved pituitaries).

In this Series, six experiments were performed, out of which two experiments 78-1, 78-2 were performed with the22 year old pituitaries and the rest 4 experiments 78-3, 78-4, 78-5 and 78-6 were performed with freshly preserved pituitaries. These experiments were performed in the second half of April and May. The data has been compiled in Table VII. All the experiments gave positive result and the dosage being 0.07, 0.08, 0.10 mg per gm of the body weight.

In all experiments, the eggs obtained by stripping were fertilized in sperm suspension and allowed to develop for further observations.

4. EXPERIMENTS PERFORMED WITH MAMMALIAN PITUITARY EXTRACTS (GOAT PITUITARY, SHEEP PITUITARY POWDER, HUMAN CHORIONIC GONADOTROPHIN), ANTUITRIN-S AND FISH PITUITARY EXTRACTS.

15 experiments were performed (2 with goat pituitary, 2 with sheep pituitary powder, 2 with human chorionic gonadotrophin, 8 with antuitrin-S and 1 with fish pituitary extract) at different dosages in the

TABLE VI

Experiments on Induced breeding in Rana limnocharis with homoplastic pituitaries preserved in absolute ethyl alcohol for two years.

Date	Experiment Number	Room Temperature	Weight of injected female (gm)	Number of pituitaries injected.	Amount of distilled water injected. (ml)	Total weight of pituitaries. (mg)	Weight of pituitaries mg per gm of the body weight of the receipient	Time of injection. (Hours)	Time of stripping next day. (Hours)	Number of eggs stripped.
April 1, 1977	77-a	18.5°C	15.1	6 (♀)	0.5	1.0	0.06	12.15	12.30	Nil
"	77-b	18.5°C	15.1	5 (♀)	0.5	1.0	0.06	12.55	12.00	490
April 9, 1977	77-c	17.0°C	17.8	5 (♀)	0.5	1.8	0.1	13.30	3.30	1059
"	77-d	17.0°C	15.0	4 (♀)	0.5	1.4	0.09	15.30	14.00	Nil
"	77-e	17.0°C	12.0	6 (♀)	0.6	1.2	0.1	16.00	15.00	Nil
April 12,1977	77-f	16.0°C	18.80	6 (♀)	0.5	1.3	0.06	11.35	11.00	91
"	77-g	16.0°C	21.00	7 (♀)	0.5	2.0	0.09	12.15	9.00	860
April 19,1977	77-h	20.0°C	20.00	6 (♀)	0.5	1.9	0.09	12.00	12.00	960

TABLE VII

Experiments on Induced breeding in *Rana limnocharis* with homoplastic pituitaries preserved in absolute ethyl alcohol.

Date	Experiment Number	Room Temperature	Weight of injected female (gm)	Number of pituitaries injected.	Amount of distilled water injected. (ml)	Total weight of pituitaries. (mg)	Weight of pituitaries mg per gm of the body weight of the receipient	Time of injection. (Hours)	Time of stripping next day. (Hours)	Number of eggs stripped.
April 23,1978	78-1	20.0°C	13.1	*6 (♀)	0.5	1.1	0.08	14.00	11.00	358
April 27,1978	78-2	21.0°C	11.1	*6 (♀)	0.5	1.2	0.10	14.30	11.45	263
May 5, 1978	78-3	23.0°C	15.0	**6 (♀)	0.5	1.2	0.08	19.30	11.20	730
May 7, 1978	78-4	22.0°C	14.0	**6 (♀)	0.5	1.1	0.07	14.00	11.45	670
May 8, 1978	78-5	24.0°C	16.0	**6 (♀)	0.5	1.3	0.08	13.00	11.30	1179
May 25,1978	78-6	24.0°C	10.0	**6 (♀)	0.5	1.0	0.10	16.00	11.00	30

months of March and April, 1978. None of the experiments gave positive result, showing that Rana limnocharis does not respond to the mammalian and fish pituitary extracts.

From the results of the experiments described above, it can be concluded that soon after the hibernation period, the frogs weighing above 16.00 gm responded to the dosage of 0.07 or 0.08 mg of the pituitary per gram of the body weight; but later mostly 0.10 mg of the pituitary per gram of the body weight (roughly 6 to 7 male or 5 to 6 females pituitaries) yielded successful results.

DISCUSSION

The induction of ovulation by the injection of the pituitary glands in anurans was first experimented by Wolf (1929) on Rana pipiens. Houssay, Guitsi and Lascano - Gonzalez (1929) confirmed this in Bufo marinus. Rugh (1934 and 1935a) performed similar experiments on the anurans of North America and he prescribed specific dosage as, number of pituitaries to be injected for different species and the technique to secure fertilized eggs. He demonstrated that mammalian pituitary extracts were effective on toads but not on frogs. In 1939, he described a technique for dissecting

and collecting the anterior pituitary in Rana pipiens and other anurans. He reported that frogs collected from hibernation could be induced to ovulate by anterior pituitary injection much before its actual breeding period. Adams and Granger (1938) described that there was no zoological specificity of gonad stimulating hormone. Ramaswami and Lakshman (1959) while working upon three frogs species found in India, viz, Rana tigrina, Rana cyanophlyctis and Rana hexadactyla showed that mammalian hormones such as follicle stimulating hormone, luteinizing hormone, pregnant mare serum, chorionic gonadotrophin, estrogen, adrenocorticotrophic hormone, cortisone and somatotrophic hormone, stimulate ovulation in combination with threshold homoplastic pituitary gland dose. Alikunhi et al (1960) and Choudhuri (1960a and 1963), while working on the induced breeding in fishes, prescribed a technique of preservation for fish pituitaries in absolute ethyl alcohol and determination of dosage of pituitary in terms of per gram of the body weight of the fish to be induced. This technique is being followed by fish farmers in India and abroad (Jhingran, 1975).

The experiments in the present investigation on the induced breeding of Rana limnocharis with homoplastic pituitaries preserved in absolute ethyl alcohol (freshly preserved in experiment Nos. 76-6, 76-7, 76-8, 76-9, 76-10,

76-11, 76-12, 76-13 and 76-14; preserved for one year in experiment Nos. 77-A, 77-B, 77-C, 77-D, 77-F, 77-G, 77-H and 77-I; preserved for two years in experiment Nos. 77-a, 77-b, 77-c, 77-d, 77-e, 77-f, 77-g and 77-h) as well as the heteroplastic pituitaries of Bufo melanostictus preserved in absolute ethyl alcohol (experiment Nos. 76-1, 76-2, 76-3 and 76-4) yielded successful results. It is possible that pituitaries preserved in absolute ethyl alcohol for three years or more may also induce ovulation successfully in frogs. Experiments with fresh pituitaries were not as convenient as with preserved pituitaries. Acetone preserved pituitaries did not yield successful results.

The procedure followed in the present investigation is modified as compared to that prescribed by Rugh (1934) and 1962). According to this technique the homoplastic pituitaries are dissected and preserved in absolute ethyl alcohol. A homogenate of these preserved pituitaries as per the calculated dosage (in terms of per gram of the body weight of the female) in 0.5 ml of distilled water is injected in the dorsal lymph sac of the female. According to Rugh's (1962) technique one has to collect a number of frogs, decapitate some of them to collect required number of pituitaries and then injecting them along with some distilled water in the abdomen of the

female. This technique is somewhat cumbersome in comparison to the technique evolved in the present investigation as described above. According to the present technique, a stock of preserved pituitaries is built up and used as and when required. The dissection of the pituitaries should be done in absolute ethyl alcohol rather than in water which reduces the chances of the hormone diffusing out at the time of the dissection. If homoplastic pituitaries are not available, heteroplastic pituitaries can also be used. Their dosage may be higher as demonstrated by Rugh (1962) and also in the present investigation. So far as dosage is concerned, in the present investigation 0.07mand 0.08 mg of the alcohol preserved homoplastic pituitary per gram of the body weight of the female Rana limnocharis yielded successful results at the beginning of the breeding season, whereas a dosage of 0.10 mg per gm (roughly 6 to 7 male or 5 to 6 female preserved pituitaries) of the body weight of the females induced ovulation successfully throughout the breeding season in the females weighing mostly above 12 gms provided they had mature ova.

Rugh (1934 and 1935a) found that mammalian pituitary extracts were not effective in frogs. In 1961, Wright and Flathers showed that injection of the frog pituitary in association with progesterone induced

complete ovulation in Rana pipiens. Ramaswami and Lakshman (1959) demonstrated that Rana cyanophlyctis responded to mammalian hormones in association with homoplastic pituitaries. In Xenopus it is a common practice to induce ovulation by mammalian hormones (Nieuwkoop and Faber, 1967). In the present investigation, Rana limnocharis did not respond to mammalian hormones. Earlier Wills, Riley and Stubbs (1933) obtained ovulation in toads by fish pituitaries. Ramaswami and Lakshman (1959) reported that Rana cyanophlyctis responds to Heteropneustes pituitaries. In the present investigation, Rana limnocharis did not respond to majpr carp pituitary extract supplied by the Central Inland Fisheries Research Institute, Barrackpore although it is commonly used in fishes.

The technique of induced breeding evolved in the present investigation suggests building up of a stock of frog pituitaries (preferably homoplastic) preserved in absolute ethyl alcohol and injecting the homogenate of these preserved pituitaries as per the previosuly determined dosage (0.10 mg per gm of the body weight of the female in Rana limnocharis), in the female frogs during their breeding period as described above. The findings reported in Chapter IV showed that gonadotropic activity of the pituitary of Rana limnocharis is at its peak from late March upto August. This period (April to

August) is the active breeding period of the frog and it is during this period that induced breeding is most effective. In our laboratory this technique has been found to be successful in Rana cyanophlyctis also. The technique can be conveniently adopted for induced breeding in frogs in any laboratory in India or abroad.

SUMMARY

The experiments on the induced breeding of Rana limnocharis Wiegmann showed that this species responds only to homoplastic or heteroplastic pituitary glands; and does not respond to major carp or mammalain pituitary. The technique of induced breeding, developed during the course of present investigation, involves building up of a stock of homoplastic pituitary glands preserved in absolute ethyl alcohol which are used in required dosage for inducing ovulation. Such preserved pituitaries were found to retain their potency up to 3 years and were as effective as the fresh ones. 0.07 - 0.08 mg of these preserved pituitaries/mg weight of the female gave successful results during early breeding period in the females of 16.00 or 17.00 gms size, whereas a dosage of 0.10 mg gave successful results throughout the breeding period in the females above 12 gms provided they had mature ova. The eggs were stripped on the subsequent day in a sperm suspension in 10% Holtfreter's solution for fertilization and the developing embryos were maintained in culture for further studies.

REFERENCES

Adams, A.E. 1931. Induction of ovulation in frogs and toads. Proc. Soc. Exp. Biol. and Med. 28: 677-681.

Adams, A.E. and B. Granger. 1938. Induction of ovulation in Rana pipiens by pituitaries of Triturus viridescens. Proc. Soc. Exp. Biol. and Med. 38: 552-553.

Alikunhi, K.H., M.A. Vijayalakshman and K.H. Ibrahim. 1960. Preliminary observations on the spawning of Indian carps, induced by injection of pituitary hormones. Ind. J. Fish. 7(1): 1-19.

Alonso - Bedate, M. and A.T. Serrano. 1979. Factors involved in experimental ovulation and fertilization in Rana ridibunda. Pol. R. Soc. Espan. Hist. Nat., Sec. Biol. 68(1-2): 25-31.

Barr, W.A. and B.M. Hobson. 1967. Method for estimating the number of eggs laid by Xenopus laevis in response to the injection of gonadotrophin. Nature 214(5090): 827-828.

Chaudhuri, H. 1960. Experiments on induced spawning of Indian carps with pituitary injections. Indian J. Fish. 7(11): 20-49.

Chaudhuri, H. 1963. Induced spawning of Indian carps. Proc. nat. Inst. Sci. India. 29B(4): 478-487.

Creaser, G.W. 1942. Ovulation induced in Rana pipiens by bird pituitary preparations. Anat. Rec. 84: Suppl. 70.

Creaser, G.W. and A. Gorbman. 1935. Apparent specificity of induced ovulation reaction in amphibia. Am. Jour. Physiol. 113: 32 pp.

Hock, L.S. and C.T. Wen. 1970. Artificial breeding and early development of the tadpoles of Rana limnocharis Boie. J. Singapore. Nat. Acad. Sci. 2(2): 59-67.

Holtfreter, J. 1931. Uber die Aufzucht isolierten Teile des AmphibienKeims. Arch. EntwMech. Org. 124: 404-466.

*Houssay, B.A., L. Guisti and J.M. Lascano-Gonzales. 1929. Implantation d'hypophyse et stimulation des Glandes et des fonctions sexuelles du Crapand. Comp. rend. Soc. de Biol. 102: 864.

Jhingran, V.G. 1975. Fish and Fisheries in India. Hindustan Publishing Corporation (India) Delhi.

Landgrebe, F.W. and G.L. Purser. 1941. Breeding of Xenopus in the laboratory. Nature. 148: 115.

Nieuwkoop, P.D. and J. Faber. 1967. Normal table of Xenopus laevis (Daudin). North Holland Publishing Company. Amsterdam.

*Ochse, W. 1968. Die Zucht des Sudafrikanischen Krallenfrosches Xenopus laevis Daudin. Gynaecologia. 126: 57-77.

Ramaswami, L.S. and A.B. Lakshman. 1958. Ovulation induced in frog with mammalian hormone. Nature. London. 181: 1210.

Ramaswami, L.S. and A.B. Lakshman. 1959. The skipper frog as a suitable embryological animal and an account of the action of mammalian hormones on spawning the same. Proc. nat. Inst. Sci. India. 25(2): 68-79.

Robinson, T.W. and H.C. Hill, Jr. 1942. Studies on induced ovulation in Rana pipiens. Proc. Fed. Am. Soc. Exp. Biol. 1: 73.

Rugh, R. 1934. Induced ovulation and artificial fertilization in the frog. Biol. Bull. 66: 22 pp.

Rugh, R. 1935a. Ovulation in the frog I. Pituitary relations in induced ovulation. II Follicular rupture to fertilization. Jour. exp. Biol. 71: 149-193.

Rugh, R. 1935b. Pituitary - induced sexual reactions in the Anura. Biol. Bull. 68: 74-81.

Rugh, R. 1939. Reaction of intact pituitary gland to artificially induced ovulation. Proc. Soc. Exp. Biol. and Med. 40: 132-136.

Rugh, R. 1948. Experimental Embryology: A manual of Techniques and Procedures. Minneapolis. Burgess Publishing Company. (III Revision, 1962).

Wills, I.A., G.M. Riley and E.M. Stubbs. 1933. Further experiments on the induction of ovulation of toads. Proc. Soc. Exp. Biol. and Med. 80: 784-786.

Wolf, O.M. 1929. Effect of daily transplants of anterior lobe of pituitary on reproduction of frog. (Rana pipiens). Proc. Soc. Exp. Biol. and Med. 26: 692-693.

Wright, P.A. 1945. Factors affecting in vitro ovulation in frogs. J. exp. Zool. 100: 565.

Wright, P.A. 1950. Time relationships in frog ovulation. J. exp. Zool. 114: 465-474.

Wright, P.A. and F.L. Hisaw. 1946. Effect of mammalian pituitary gonadotropins on ovulation in the frog, Rana pipiens. Endocrinol. 39(4): 247-255.

Wright, P.A. 1960. Experiments with ovulation induced in vitro by means of steriods in frogs and marine fishes. Biol. Bull. Woods. Hole. 119: 351.

Wright, P.A. and A.R. Flathers. 1961. Facilitation of pituitary induced frog ovulation by progesterone in early fall. Proc. Soc. Exp. Biol. and Med. 106: 346-347.

* Not consulted in original.

Chapter 6

Spawn Size

Fertility Ratio

Embryonic Survival and Mortality

** Influence of Limiting temperatures on Development

** Paper published

Roy, D. and M.K.Khare. 1979. The influence of embryonic limiting temperatures on the development of Rana limnocharis Wiegmann. Biol. J. Linn. Soc. 11(3): 279-288.

INTRODUCTION

The present chapter deals with the following ecological aspects related to embryonic and postembryonic development of Rana limnocharis Wiegmann studied under laboratory condition. (1) Spawn size (2) Fertility ratio. (3) Survival and mortality. (4) Influence of temperature on development. A comparative statement of the spawn size of many frog species has been given by Rugh (1962). There appears to be no specific data on spawn/clutch size for frog species available in our country. However, the fecundity of three Indian frog species has been described; Rana tigrina 3,000-20,000 eggs, Rana crassa 2,000-6,000 eggs and Rana hexadactyla 1,000-9,000 eggs depending upon the body size (Mondal, 1975). In the present investigation data on the spawn size of induced bred Rana limnocharis has been collected during the breeding seasons (1976, 1977 and 1978). During the course of investigation on experimental breeding and preparation of developmental table of this frog species, it was experienced that a small number of eggs (20-50 eggs per 200 ml of sperm suspension) can be easily fertilized and developed. In this chapter an analysis of the six experiments on fertility percentage of complete spawns in the same amount of sperm suspension have been included and percentage of survival and mortality of experimentally bred embryos has been worked out. There

are very few contributions available on these aspects (Woodruff, 1976) as it is difficult to make such investigations in the natural condition. Temperature has been found to be another important factor governing the development, growth and biogeographic distribution of anurans (Zweifel, 1968; Brown, 1975a and 1975b). In the present investigation, therefore, lower and upper limiting temperatures of Rana limnocharis were worked out during early embryonic stages and their influence on the development and growth of the embryonic as well as post-embryonic stages has been investigated. A fundamental knowledge of all these aspects, viz, spawn size, fertility ratio of the eggs, survival and mortality of developing embryos and influence of limiting temperatures on development is required for successful induced breeding and culture not only of Rana limnocharis but also of other anurans.

REVIEW OF LITERATURE

A. Spawn size, Fertility ratio, Survival and Mortality.

As Calef (1973) observed, "Although the tadpoles of frogs and toads are among the most common inhabitants of lakes, ponds and streams, little is known about their population dynamics or other ecological roles in these

communities" holds true for fertility ratio, survival and mortality of all early developmental stages of anurans. There are few contributions on these aspects. Bragg and Bressler (1951) appear to be the first workers to have investigated the viability of eggs of Bufo cognatus. It lays 20,000 ova. 66% of the ova either were not fertilized or failed during the embryonic development. Knoepffler (1962) reported Discoglossus pictus lays 800 eggs at a time and stated that the speed with which the eggs were released and low motility of the sperms were responsible for low fertility percentage. Herreid and Kinney (1966) investigated the survival of the larvae of Alaskan woodfrog, Rana sylvatica and obtained the average fertilization success of 86% and embryonic mortality through gastrulation of 4%. Inger and Bacon (1968), while working on the six Bornean species of rain forest frogs from Sarawak, described that the clutch size of Rana species (Rana blythi, Rana ibanorum, Rana macrodon and Rana hosei) were smaller than those of temperate zone species. In Bufo asper there was no difference in clutch size. Bogert (1969) reported the eggs and hatchlings of the Mexican leptodactylid frog, Eleutherodactylus decoratus. Three clutches contained 29 to 32 eggs. The batches had survivorship of 100%, 0% and 60%. Van Gelder and Oomen (1970) working in Netherlands on Rana arvalis reported fungal infestations among the

spawns in natural condition. Kozlowska (1971) while studying the reproductive biology of mountain and lowland common frogs, Rana temporaria from Poland noted that the amount of spawn was lower, the number of eggs lower and the size of the eggs larger in frogs in mountain habits in comparison to those of the lowland. In 1972, Izvanova studied the growth and development of Anura under experimental conditions. He reported that the larvae of Pelobates fuscus in aquarium (10 larvae in 10 litre of water) markedly decreased the larval developmental period, accelerated metamorphosis which reduced the size of the tadpoles in comparison to the larval development in lakes and ponds under natural conditions. Calef (1973) investigated in detail the survivorship, distribution, growth rates and normal predation rates on the population of Rana aurora tadpoles in British Columbia and reported 95% mortality. Licht (1974) worked on the survival of embryos, tadpoles and adults of Rana aurora aurora and Rana pretiosa pretiosa. He reported that predators, food and climatic conditions, such as excessive cold or dryness were responsible for the mortality. Cooke in 1975 while working upon the spawn site selection and colony size of the frog Rana temporaria and the toad Bufo bufo reported that fungus was responsible for the low percentage of hatchability. In 1975, Koskela and Pasanen working in Finland, on the reproductive biology of the common frog

Rana temporaria noted that the total volume of spawns, size and number of the eggs were dependent on the size of the female. The production of spawn decreases as a result of the shorter eating period. Recently in 1976, Woodruff seems to have investigated the problem of embryonic mortality thoroughly for Pseudophryne (Anura: Leptodactylidae). He recorded Pseudophryne bibroni, Pseudophryne dendyi and Pseudophryne semimarmorata laid batches of 70 to 90 ova. Of these 98-100% ova were fertilized but mortality less than 5% occurred due to fungal infestations and most failures occurred during gastrulation. Commenting on the fertilization of immature eggs of Rana pipiens, Elinson (1977) reported that they can be fertilized to a low frequency when eggs were inseminated by high concentration of sperms in the presence of water extract of jellied eggs.

Crowding seems to affect growth, development and mortality of anuran embryos and larvae. Lynn and Edelman (1936) showed that crowding not only affects the rate of development but the success of achieving metamorphosis. They established a ratio at the pre-feeding stage, as 1 tadpole per 2 cc of medium in a total of 50 cc per finger bowl. At feeding stage this would result in crowding and development retarded. Guyetant (1970) gave an evidence of group effect upon the growth and metamorphosis of the midwife toad, Alytes obstetricans. There is

a recent report by Shvarts and Pyastolova (1970) on the effects of metabolities of tadpoles of Rana macronemis and Rana arvalis showed that, the metabolities excreted are not inhibitors, as commonly considered, but regulators of development for the organism and at population level.

B. Influence of temperature on development.

Among various ecological factors, temperature adaptation appears to be one of the important characteristics of amphibians and related directly to their geographic distribution breeding habits and developmental rates as reviewed by Zweifel (1968), Bachmann (1969) and McLaren and Cooley (1972). Atlas in 1935, demonstrated that Rana pipiens embryos become increasingly tolerant with age to high temperatures. While studying this factor on certain North American frogs, Moore (1939 and 1942) found that temperature adaptation of embryos correlates well with their distribution. DuShane and Hutchinson (1941) felt that genetic differences may be responsible for the distribution of a species. Ivanova and Vinogradov (1955) found that temperature adaptation may account for metamorphosis also. Herreid and Kinney (1967) did not observe any difference in development of Rana sylvatica embryos at fluctuating or constant temperatures. Ballinger and McKinney (1966) and Licht (1971) investigated lower and upper lethal temperatures of certain species

of frogs. Zweifel (1968) exhaustively investigated the reproductive biology and embryonic temperature tolerance, in nine anuran species inhabiting desert grasslands and arid uplands and adjacent New Mexico. He defined lethal temperatures as these below which (lower lethal) or above which (upper lethal) fewer than 50% of the embryos develop normal external morphology. He studied only embryonic temperature tolerance on the belief that it was reasonable to assume that embryos attaining Stage 20 in normal condition will continue to develop normally. Cross and Hoyt (1927) worked on the effect of combined temperature and photoperiod on early development rate of Rana pipiens eggs. He showed that the effect of temperature on early development of eggs, appears to be directly and linearly related and not markedly effected by photoperiod. While working upon the embryonic temperature adaptation and genetic compatibility of two allopatric populations of Scaphiopus hammondi, Brown (1967) reported that high temperature tolerance is influenced by the stage of development. In 1975a, he investigated that Ascaphus truei have narrow temperature tolerance range and slow rate of development. In the same year, he investigated the embryonic temperature adaptations and distribution of two widely separated populations of Hyla regilla in relation to their environment. Besides his own findings, he has summarised the limiting temperatures of many anuran

species and discussed their geographic and ecological distribution. McLaren and Cooley (1972) analysed the embryonic temperature adaptation of 14 ranid species from North America, Europe and Japan, by applying Belehradek's (1935) temperature function. Kuramoto (1975) while working on 12 anuran species in Japan, also attacked the problem by applying Belehradek's temperature function and showed that embryos of frogs which breed and hatch in summer or warm waters are smaller and consume less oxygen than these which breed in winter or cold water. Metabolic rate is higher and dissolved oxygen is less in warm water than in cold.

Among Indian frog species, the only report on embryonic temperature tolerance is on Rana cyanophlyctis (Dasgupta and Grewal, 1968 and 1970). They reported that its population from Northern India has lower and upper limiting temperature of 22°C and 31.5°C respectively, but a population from Southern India has a lower limiting temperature of 17°C to 18°C.

MATERIALS AND METHODS

The study on spawn size, fertility, survival and mortality and influence of temperature on development was made together with experiments on induced breeding

of Rana limnocharis. On a few occasions the natural spawn size was studied, but mostly number of eggs in each spawn stripped during induced breeding experiments was counted for investigating the fertility ratio. Each female was stripped in the same amount of sperm suspension, prepared by macerating 2 testis from mature males in 200 ml of Holtfreter's solution and the number of eggs undergoing first cleavage was recorded. The eggs which did not show any cleavage were taken as unfertilized and removed. For studying the survival and mortality the cleaving eggs were transferred in different culture dishes. The culture medium was not changed like earlier experiments (where culture medium was changed regularly and 100% survival was obtained) as such change is not always possible in the naturalpand condition. For investigating the embryonic temperature tolerance, groups of 20 embryos at the first cleavage stage were transferred to finger bowls containing 200 ml of 10% Holtfreter's solution in each. In some preliminary pilot experiments embryonic temperature tolerance was determined by exposing the fertilized eggs at first cleaving stage to constant high and low temperatures. The lower limiting temperature was found to be (5°C), room temperature (14°C to 22°C) and the upper limiting temperature (28°C). Lethal temperatures are defined as those below which (lower lethal) or above which (upper lethal) fewer than

50% of the embryos develop normal external morphology (Zweifel, 1968; Brown, 1975). At various stages, sizes of the larvae and the time required for their development were recorded. Observations were made on 5 replicates in each case. At the time of hatching some algae was added to the culture medium in all bowls as food for the larvae.

OBSERVATIONS

1. SPAWN SIZE.

More than 40 successful induced breeding experiments were carried out during the course of present investigation. Out of these the number of eggs were counted only in 28 experiments. The data have been presented in Table I. The smallest number of eggs obtained were 30 and the largest number of eggs obtained were 1,462. In 14 cases, the number of eggs per spawn ranged from 30 to 500; 9 cases from 500 to 1,000; and in 5 cases from 1,000 to 1,462. On 3 occasions (July, 1975; August, 1975; April, 1976) the spawns were collected from ponds. The number of eggs in these spawns was 700, 500, 600 respectively. From females weighing 12 gm or less number of eggs obtained in all experiments was below 300; and from females weighing above 12 gm more than 300 eggs were obtained in every experiment.

TABLE I

Spawn size of *Rana limnocharis* Wiegmann (after induced breeding)

Date	Weight of famale (gm)	Number of ova obtained.	Date	Weight of female (gm)	Number of ova obtained
April 26, 1976	15.0	178	April 14, 1977	12.0	110
"	12.0	189	April 19, 1977	15.0	840
"	12.0	182	"	15.0	1362
May 5, 1976	12.0	265	"	20.0	960
"	15.0	182	April 26, 1977	20.5	1457
May 11, 1976	12.0	289	May 3, 1977	17.0	590
June 16, 1976	10.0	167	May 5, 1977	17.0	780
April 1, 1977	15.0	780	"	16.5	840
April 5, 1977	17.8	490	April 23, 1978	13.1	358
"	15.0	1059	May 6, 1978	15.0	730
April 12, 1977	18.8	91	May 7, 1978	14.0	670
"	16.0	472	May 8, 1978	16.0	1119
"	21.0	860	May 25, 1978	10.0	,30
April 14, 1977	14.3	1462	July 24, 1978	11.1	263

Out of 8 cases, where the weight of female was 12 gm or less, eggs obtained were less than 200 in 5 cases and more than 250 in 3 cases. From females weighing above 14 gms eggs obtained mostly were between 500 to 1,000 in each experiment. The number of eggs so obtained during each experiment was always more in the month of April than in later months.

2. FERTILITY RATIO.

While studying experimental breeding and artificial insemination and developmental table it was experienced that batches of 20 to 50 eggs were easily fertilized in sperm suspension prepared by macerating 2 testis from mature males in 200 ml of Holtfreter's solution. In the present investigation, 6 experiments were performed to see the fertility of ratio of eggs, when all eggs from one female was stripped in the same amount (200 ml) of sperm suspension prepared as described above. In these experiments the number of ova fertilized were 58.47%, 77.61%, 35.74%, 93.57%, 99.23% and 100.00%. The data has been compiled in Table II. It was noted that, if the stripping was done very fast as in experiment Nos. 1, 2 and 3, the fertility ratio was found to be very low. In experiment No. 3, the number of eggs stripped was 1,119 but only 400 eggs were fertilized. This shows that all eggs were not fully exposed to spermatozoa. In contrast to these experiments, the experiment No. 4 shows higher

TABLE II

Fertility ratio in Rana limnocharis Wiegmann

Experiment number.	Amount of sperm suspension (ml)	Number of ova stripped	Number of ova fertilized.	Percentage of fertilized eggs.	Method of stripping.
1	200	171	100	58.47	quick
2	200	670	520	77.61	quick
3	200	1119	400	35.74	quick
4	200	358	335	93.57	slow
5	200	263	261	99.23	very slow
6	200	320	320	100.00	very slow

fertility ratio, where the rate of stripping was comparatively slower. In experiment Nos. 5 and 6, the stripping was done very slowly so as to allow each egg sufficient time for exposure to spermatozoa in the sperm suspension and in both the cases fertility ratio was above 98%. These experiments show that the eggs should be stripped in the viable sperm suspension slowly in order to get higher fertility ratio.

3. SURVIVAL AND MORTALITY OF THE EMBRYOS.

The survival and mortality of the embryos of Rana limnocharis was worked out both in 10% Holtfreter's solution and in pond water up to the stage of hatching. The same culture medium was maintained up to hatching in each experiment unlike the earlier experiments where it was changed regularly. The data has been tabulated in Table III.

A. Development in Holtfreter's solution.

5 sets of experiments were performed to find out the survival and mortality of the embryos in this culture medium. In every case embryos were transferred at "Two cell Stage".

Experiment Set No. 1.

The embryos were kept in 3 dishes. In dish No. 1a, out of 52 embryos 22 died and 30 hatched. In dish No. 1b, out of 55 embryos 23 died and 32 hatched. In dish No. 1c,

TABLE III

Survival and Mortality of Rana limnocharis Wiegmann embryos under laboratory conditions

Experiment Set Number.		Culture dish Number	Room temperature. (°C)	Number of embryos at I cleavage transferred in each dish.	Number of embryos hatched	Number of embryos died	Percentage of mortality.	Culture medium.	Remarks
A.	1	1a	14-22	52	30	22	42.30	10% Holtfreter's solution	Culture medium remained clean.
		1b	"	55	32	23	41.81	"	"
		1c	"	63	34	29	46.03	"	"
	2	2a	"	63	49	14	22.22	"	"
		2b	"	92	9	83	90.21	"	Fungal infection was observed.
	3	3	"	100	55	45	45.00	"	Culture medium became turbid.
	4	4a	"	70	63	7	10.00	"	Culture medium remained clean.
		4b	"	72	54	18	25.00	"	"
		4c	"	80	61	19	23.75	"	"
		4d	"	84	67	17	20.23	"	"
		4e	"	200	21	179	89.5	"	Culture medium became turbid.
		4f	"	301	37	264	87.70	"	"
	5	5	"	51	14	37	72.54	"	Fungal infection was observed.
B.	6	6a	"	56	34	22	39.28	Pond water.	Culture medium remained clean.
		6b	"	59	30	29	49.15	"	"
		6c	"	61	34	27	36.98	"	"

out of 63 embryos 29 died and 34 hatched. The culture medium remained clear for all 6 days and there was no apparent contamination.

Experiment Set No. 2.

In this experiment the embryos were divided in 2 culture dishes 63 in one and 92 in other. In dish No. 2a, out of 63 embryos 14 died and 49 hatched. In dish No. 2b, out of 92 embryos 83 died and 9 hatched. In this dish fungal infection was observed.

Experiment Set No. 3.

In this experiment all 100 embryos in the "Two cell Stage" were transferred in the same dish No. 3. Out of 100 embryos 45 died and 55 hatched. The culture medium was observed to have become turbid.

Experiment Set No. 4.

In experiment Set No. 4 the cleaving embryos at "Two cell Stage" were kept in 6 culture dishes in increasing numbers from 70 to 301. In dish No. 4a, out of 70 embryos, 7 died and 63 hatched. In dish No. 4b, out of 72 embryos 18 died and 54 hatched. In dish No. 4c, out of 80 embryos 19 died and 61 hatched. In dish No. 4d, out of 84 embryos 17 died and 67 hatched. In dish No. 4e, out of 200 embryos 179 died and 21 hatched. In dish No. 4f, out of 301 embryos 264 died and 37 hatched.

In culture dishes 4e and 4f, the culture medium was observed to have become turbid.

Experiment Set No. 5.

In this experiment, 51 cleaving embryos at "Two cell Stage" were kept in dish No. 5, out of these 37 died and 14 hatched. The culture was infected with fungal infection.

B. Development in Pond water.

One set of experiment in 3 culture dishes was performed in pond water. In dishes No. 6a, 6b and 6c, out of 56 embryos 22 died and 34 hatched; out of 59 embryos 29 died and 30 hatched; out of 73 embryos 27 died and 34 hatched respectively. The culture medium remained clear till the time of hatching.

Average percentage of mortality of embryos in 10% Holtfreter's solution was 47.40% and pond water 31.80%. The percentage of mortality was observed to be very high whenever the culture medium was infected as in culture dishes No. 2b, 3, 4e, 4f and 5.

4. INFLUENCE OF TEMPERATURE ON DEVELOPMENT.

In this investigation effect of lower (5°C) and upper (28°C) embryonic limiting temperature and room temperature (14°C to 22°C) has been studied on the development of embryonic and post embryonic developmental

stages of Rana limnocharis. In the natural condition the lowest water temperature during the breeding season was 10°C and highest temperature was 24°C. The observations were made under two headings :

1. Temperature and Development (Table IV).
2. Temperature and Size (Table V; Fig. 1).

1. Temperature and Development.

Before starting this investigation it was determined that, embryonic mortality below 5°C and above 28°C was about 50%. As such these temperatures were taken as lower and upper embryonic temperature tolerance limits (as prescribed by Zweifel, 1968) for the development of Rana limnocharis. At these temperature more than 50% embryos developed normal external morphology. Table IV, shows influence of 3 selected ranges of temperature on development.

(i) Room temperature incubation (14°C to 22°C).

If "Two cell Stage" taken as representing 0 Hour development time, then the embryos hatch at about 76 Hours. The hatched larvae measure about 0.85 cm in length of which trunk length was 0.35 cm and tail length was 0.50 cm. The hind limb buds appeared at 464 Hours, became fully developed by about 672 Hours and metamorphosis was completed by 864 Hours. Some mortality was observed after Stage 19 but 80% embryos reach the froglet stage.

TABLE IV

Time required (Hours) to reach specific developmental stage and percentage of survival of Rana limnocharis Wiegmann embryos and larvae.

Developmental Stage	Low temperature 5°C		Room temperature 14°C-22°C		High temperature 28°C	
	Time (Hours)	Survival	Time (Hours)	Survival	Time (Hours)	Survival
EMBRYONIC STAGES						
3 2-cell	0	100%	0	100%	0	100%
4 4-cell	0.75	100%	0.50	100%	0.25	100%
5 8-cell	2.25	100%	1.00	100%	0.75	100%
6 16-cell	4.25	100%	1.75	100%	1.25	100%
7 32-cell	7.50	100%	2.00	100%	1.75	100%
8 Mid-cleavage	12.00	100%	2.75	100%	2.25	100%
9 Late cleavage	17.25	100%	7.50	100%	4.25	100%
10 Dorsal lip	26.75	100%	13.00	100%	5.75	100%
11 Mid gastrula	45.75	100%	17.25	100%	7.50	100%
12 Late gastrula	53.25	100%	21.25	100%	9.25	100%
13 Neural plate	119.75	100%	25.75	100%	13.00	100%
14 Neural fold	142.00	100%	28.75	100%	17.25	100%
15 Rotation	165.00	100%	34.25	100%	19.50	100%
16 Neural tube	194.00	75%	44.75	100%	21.25	100%
17 Tail bud	216.00	75%	49.25	100%	24.25	100%
18 Muscular response	248.00	75%	54.75	100%	32.00	100%
19 Heart beat	284.00	64%	62.25	98%	39.75	90%
20 Gill circulation	332.00	64%	76.00	98%	46.75	90%
LARVAL STAGES						
21 Mouth open	396.00	64%	91.00	98%	120.00	90%
22 Tail fin circulation	504.00	50%	120.00	98%	216.00	90%
23 Opercular fold	625.00	25%	144.00	98%	288.00	84%
24 Operculum partially closed on right	698.00	25%	216.00	98%	456.00	84%
25 Operculum closed	768.00	16%	312.00	96%	624.00	84%
26 Hindlimb bud	All died		464.00	96%	864.00	68%
27 Hindlimb developed			672.00	92%	1248.00	68%
28 Forelimb protrusion			720.00	92%	1296.00	68%
29 Forelimb developed			768.00	92%	1392.00	68%
30 Beginning of metamorphosis			778.00	88%	1468.00	64%
31 Tail stub remained			840.00	80%	1566.00	64%
32 Young frog			864.00	80%	1672.00	50%

(ii) Low temperature incubation (5°C).

The low temperature retards the development of the embryos from the very beginning in comparison to the rate of growth at room temperature. The embryos hatched at 332 Hours, and measured about 0.6 cm in length (the trunk length 0.45 cm and the tail length 0.15 cm). The embryos were smaller in size in comparison to the embryos hatched at the other temperatures. (Room temperature and High temperature). About 20% of the embryos showed stunted growth and looked morphologically abnormal by the time they reached Stage 16 and died. About 14.7% of the survivors died at Stage 21. More mortality occurred at successive stages. The hindlimb buds appeared in 16% of the survivors at about 768 Hours but the larvae appeared abnormal and died soon after this stage.

(iii) High temperature incubation (28°C).

High temperature incubation induced faster rate of development in comparison to the other 2 sets. About 10% mortality was observed at Stage 19. The embryos hatched at about 46.75 Hours and measured about 0.95 cm. The trunk length was 0.35 cm and the tail length was 0.60 cm. They were therefore larger than the embryos hatched at lower temperatures. Although higher temperature exerted an accelerating effect up to hatching but

TABLE V

Influence of temperature on size of Rana limnocharis tadpoles.

Developmental Stage	At low temperature 5°C			At room temperature 14°C - 22°C			At high temperature 28°C		
	Total length (cm)	Trunk length (cm)	Tail length (cm)	Total length (cm)	Trunk length (cm)	Tail length (cm)	Total length (cm)	Trunk length (cm)	Tail length (cm)
*20 Gill circulation	0.60	0.45	0.15	0.85	0.35	0.50	0.95	0.35	0.60
21 Mouth open	0.90	0.65	0.25	1.25	0.50	0.75	1.28	0.50	0.78
22 Tail fin circulation.	0.95	0.65	0.30	1.45	0.60	0.85	1.40	0.60	0.80
23 Opercular fold	1.05	0.55	0.50	1.57	0.62	0.95	1.55	0.65	0.70
24 Operculum partially closed.	1.25	0.60	0.65	1.70	0.75	0.95	1.70	0.70	1.00
25 Operculum closed	1.46	0.68	0.78	1.95	0.85	1.10	1.70	0.70	1.00
26 Hindlimb bud	All died			3.00	1.25	1.75	2.80	1.10	1.70
27 Hindlimb developed				3.50	1.50	2.00	3.18	1.30	1.88
28 Forelimb profrusion				3.65	1.55	2.10	3.25	1.35	1.90
29 Forelimb developed				3.80	1.65	2.15	3.50	1.50	2.00
30 Beginning of metamorphosis.				2.55	1.55	1.00	2.25	1.25	1.00
31 Tail stub remained				1.55	1.00	0.55	1.50	1.00	0.50
32 Young frog				1.00	1.00	0.00	0.80	0.80	0.00

* Stages correspond to developmental stages of ROY and KHARE (1978)

Fig. 1 - Influence of temperature on size of the tadpoles of Rana limnocharis Wiegmann.

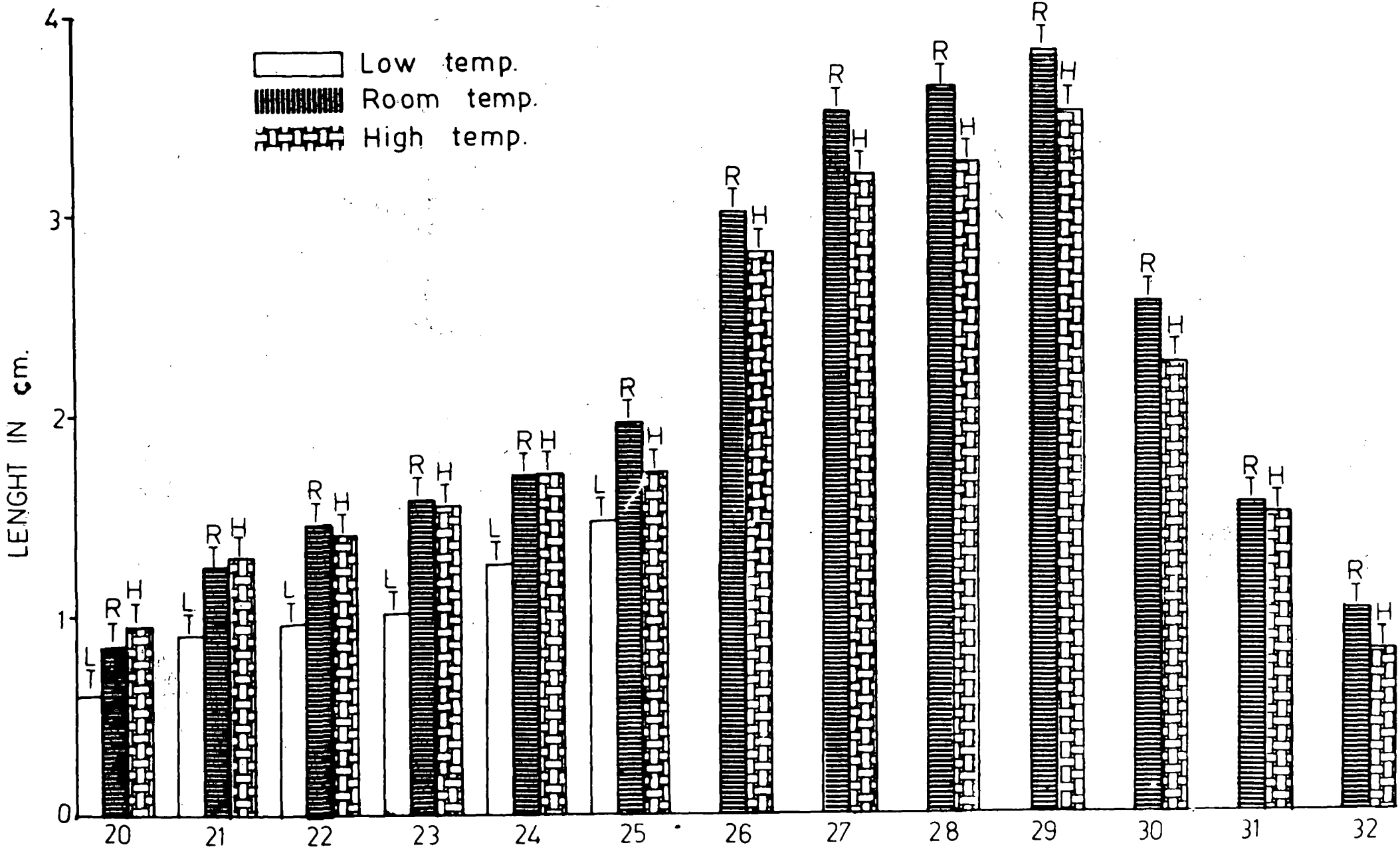

Low temp.
Room temp.
High temp.
LENGHT IN cm.
0
1
2
3
4
LT
RT
HT
20
21
22
23
24
25
26
27
28
29
30
31
32
Developmental Stage

Fig 1

after this stage the developmental rate was found to slow down. The total length of the larva at this stage was about 1.7 cm (the trunk length was 0.7 cm and the tail length was 1.0 cm) at Stage 25. There was a marked slowing of development after 456 Hours. The hind limb buds appeared by about 864 Hours and became fully developed by 1248 Hours. Metamorphosis was completed by 1672 Hours. About 10% mortality occurred at Stage 19 and it gradually increased so that only of the 50% larvae reached the froglet stage.

2. Temperature and size.

Table V and Fig. 1 shows size of tadpole at different temperatures. Embryos hatched at 5°C are smaller than those hatched at higher temperatures. The tail length in proportion to the body varies at the 3 temperature ranges. At 5°C the tail length of Stage 20 is 33% of the trunk length, whereas at room temperatures 150% and at 28°C it is 175% of the trunk length. The total size of the metamorphosed frog, however, is greatest at room temperature and less at high temperature.

DISCUSSION

EMBRYONIC DEMOGRAPHY.

Our understanding of many bioecological aspects of earliest stages of development of anurans such as spawn

size, fertility ratio and survival and mortality is still fragmen

This is partly because of the paucity of reliable quantitative techniques for investigation in natural conditions. There are many factors which govern these aspects such as predation of eggs and larvae by other animals, bacterial and fungal infections or inherant genetic and chromosomal abnormalities. Environmental factors such as temperature, light, rainfall and availability of food may also be responsible for controlling earliest stages of development. It is therefore felt that, if embryos can be developed upto larval stages or even upto froglet stage in the laboratory condition and then released in the ponds of natural habitat, the survival percentage of the frogs can be highly increased. These facts can be better understood by citing some important references in this connection. Bragg and Bressler (1951) found 882 unhatched eggs in a batch of 1331 capsules of Bufo cognatus. 66% of these ova were either not fertilized or failed during embryonic stage of development. Knoepffler (1962) investigated that, in Discoglossus pictus 60% of the eggs may not be fertilized. Although he did not give any data to support his statement but he did cite on instance where 41 out of 86 ova were fertilized and started to develop in an aquarium. He attributed the low proportion of fertilized eggs to the high speed with which the eggs

are released and low motility of the spermatozoa. Bogert (1969) observed 3 clutches of Eleutherodactylus decoratus containing 20 to 32 eggs. He recorded 100%, 0% and 60% embryonic survival. Calef (1973) recorded fingal infestations in the eggs of Rana aurora in British Columbia. Van Gelder and Oomen (1970) also noted fungal infestations among batches of eggs in nature. First careful experimental observation with a view to fill this gap towards understanding anuran demography has been made by Woodruff (1976). He investigated the embryonic mortality of Pseudophryne bibroni, Pseudophryne denyi and Pseudophryne semimarmorata. He investigated embryonic mortality in 50 batches of eggs collected from the field, each batch had 70-90 ova. The fertilization was 98-100%, embryonic mortality was less than 5%. Commenting upon the survivorship of Rana sylvatica larvae, Herreid and Kinney (1967) reported a mean mortality rate of 86%. Looking to such a high embryonic and larval mortality rate in the nature, it would perhaps be justified if embryos and larvae can be reared in clean laboratory conditions where there is no danger of predation and by careful manipulation bacterial and fungal infections can also be checked. In the present investigation as a result of induced breeding number of eggs obtained ranged from 30 to 1,462. In many cases females weighing above 13 gms produced eggs ranging from 500 to 1000. The experiments on their fertility ratio indicate that if the eggs are stripped slowly in the sperm suspension

allowing each egg sufficient time and exposure to spermatozoa 100% fertilization can be obtained. The experiments on survival and mortality show that the percentage of mortality in 10% Holtfreter's solution (47.40%) or in pond water (31.80%) remains almost similar. When the culture medium was charged everyday and number of embryos in each culture dish was less (20-50/ 200 ml of culture medium) 98% survival of embryos and 80% survival up to froglet stage at room temperature was noted (Table IV).

TEMPERATURE AND DEVELOPMENT.

Rana limnocharis is distributed in the Eastern tropics in areas with both warm humid as well as cold climates. Studies on its embryonic temperature tolerance and adaptation may help in understanding its biogeographic distribution. Zweifel (1968) gave the first very exhaustive account of adaptation of anuran embryos to temperature and correlated his findings with the distribution. According to the criteria established by Zweifel (1968) and Brown (1975a and 1975b) lethal temperatures are defined as those below which (lower lethal) or above which (upper lethal) fewer than 50% of the embryos develop normal external morphology. Zweifel (1968) says, "It is reasonable to assume that embryos attaining Stage 20 in normal condition will continue to develop normally". The Stage 20 which he took as end point corresponds to

Stage 20 in the present investigation. According to this criteria the lower and upper limiting temperatures of embryonic development for this species (Rana limnocharis) available at Shillong have been found to be 5°C and 28°C. Since conditions being similar in all the experiments performed at 3 ranges of temperatures in the present investigation, the findings that the tadpoles were unable to survive beyond Stage 26 at 5°C suggests that, limiting temperatures should not be based only on embryonic temperatures tolerance limits, rather they should be worked out for complete development including post-embryonic stages taking metamorphosis to young froglet stage as the end point. Brown (1975a and 1975b) concluded that cold adapted species have wider embryonic temperature tolerance range than warm adapted species. For Indian species of frogs the limiting temperatures have been worked out for the skipper frog, Rana cyanophlyctis by Dasgupta and Grewal(1968 and 1970). The authors found that the limiting temperatures of its North Indian populations was 22°C to 31°C; whereas for a population of Rana cyanophlyctis found in South India they were 17°C to 31°C. The limiting temperatures for Northern population appear unusual as the species is presumably warm adapted. In other species of frog studied, very low temperature incubation appears to slow down developmental rates (Olson, 1942; Brown, 1975a and 1975b). In Rana limnocharis this is also the case and

results in developmental abnormalities so that larvae do not survive long after hatching (Table IV). High limiting temperatures accelerate development during embryonic stages but inhibit development during post embryonic stages, so that the larva takes almost double the time to metamorphose into froglet stage when compared to those incubated at room temperature. Work on Bufo punctatus by Zweifel (1968) showed that high temperature have an inhibitory effect on development.

While working upon Ascaphus truei Brown (1975a) showed that a narrow temperature tolerance range (13.5°C to 18.5°C) correlates with species distribution and appears to be an adaptation for survival in relatively constant and low stream temperature. As a result of observations on Washington and Californian population of Hyla regilla Brown (1975b) showed that its embryonic temperature tolerance correlates with its abundance and wide ranging geographic and ecological distribution. Rana limnocharis is found both in cold climates at high altitudes and in the hotter plains of the Eastern tropics. This indicates that it may also have different thermal groups, that is, cold adapted and warm adapted species. Rana limnocharis available at Shillong has very large embryonic temperature tolerance range. Kuramoto (1975) studied the breeding habits of 12 species of frogs and reported that species which breed in summer or in warm

water are smaller than those which breed in winter or cold water. Lovtrup (1961) studied the embryonic development and enzyme synthesis of Rana platyrrhina at different temperatures and Somero (1969) showed a sharp decrease in enzyme substrate affinity. This frequency occurs at extremes of an organisms habitat temperature and may be important in establishing thermal tolerance limits for an organism. In the present investigation although the development of Rana limnocharis was found to be normal at 14°C to 22°C, its lower (5°C) and upper (28°C) limiting temperatures may account for its better adaptive capacity and be responsible for its varied distribution pattern. It may, however, be interesting to study the embryonic temperature tolerance of its warm adapted population in order to confirm this conclusion.

SUMMARY

Observations and results on the spawn size, fertility ratio of the eggs, survival and mortality and influence of limiting temperatures on the development of Rana limnocharis Wiegmann investigated under laboratory conditions have been included in this chapter. The study reveals that from 500 up to 1000 eggs can be obtained by induced breeding from females weighing above 13 gms. The experiments showed that when the eggs were slowly stripped in a sperm suspension allowing each egg sufficient exposure to the spermatozoa a very high percentage (more than 97%) fertilization could be obtained. The percentage of survival and mortality in 10% Holtfreter's solution and pond water remained almost similar. When the number of embryos in each culture dish was maintained low (20-50 eggs/200 ml of culture medium) with the culture medium changed everyday 98% survival of embryos and 80% survival up to froglet stage was obtained at room temperature (14°C to 22°C). The range of temperature tolerance for this species was found to be between 5°C (lower limiting temperature) and 28°C (upper limiting temperature) for embryonic as well as post-embryonic stages. With below 5°C and above 28°C, normal external morphological features were seen only in less than 50% embryos, as at very low temperatures the development slowed down resulting in developmental abnormalities; and the larvae did not survive

for long after hatching. High temperature accelerated development during embryonic stages but inhibited development during post-embryonic stages; thus the larvae took double the time to metamorphose into froglet stage, as compared to those incubated at room temperature. The wide temperature tolerance of Rana limnocharis probably explains its abundance and the wide ranging geographic and ecological distribution in both cold, sub-tropical climate at high altitudes and in the hotter plains of eastern tropics.

REFERENCES

Atlas, M. 1935. The effect of temperature on the development of Rana pipiens. Physiol. Zool. 8: 290-310.

Bachmann, K. 1969. Temperature adaptation of amphibian embryos. Amer. Natur. 103: 115-130.

Ballinger, R.E. and C.O. McKinney. 1966. Developmental temperature tolerance of certain anuran species. Jour. Exp. Zool. 161: 21-28.

*Belehradek, J. 1935. Temperature and living matter. Borntraeger, Berlin. pp 277.

Bogert, C.M. 1969. The eggs and hatchlings of the Mexican leptodactylid frog Eleutherodactylus decoratus. Taylor. Amer. Mus. Novit. 2376: 1-90.

Bragg, A.N. and J. Bressler. 1951. Viability of the eggs of Bufo cognatus. Proc. Okla. Acad. Sci. 32: 13-14.

Brown, H.A. 1967. Embryonic temperature adaptations and genetic compatibility in two allopatric populations of the spadefoot toad, Scaphiopus hammondi. Evolution. 21; 742-761.

Brown, H.A. 1975a. Temperature and development of the tailed frog, Ascaphus truei. Comp. Biochem. Physiol. 50A: 397-405.

Brown, H.A. 1975b. Embryonic temperature adaptations of the Pacific treefrog, Hyla regilla. Comp. Biochem. Physiol. 51A: 863-873.

Calef, G.W. 1973. Natural mortality of tadpoles in population of Rana aurora. Ecology. 54: 741-758.

Cooke, A.S. 1975. Spawn site selection and colony size of the frog (Rana temporaria) and the toad (Bufo bufo). J. Zool. Lond. 175: 29-38.

Cross, J.F. and R.D. Hoyt. 1972. The effect of combined temperature and photoperiod on early development rate of Rana pipiens eggs. Trans. Ky. Acad. Sci. 33(1/2): 27-32.

Dasgupta, S. and M. Grewal. 1968. The selective advantage of temperature tolerance among the progeny of frogs with vertebral fusions. Evolution. 22: 87-92.

Dasgupta, S. and M. Grewal. 1970. Inheritance of vertebral fusion in the skipper frog. J. Hered. 61: 174-176.

DuShane, G.P. and C. Hutchinson. 1941. The effect of temperature on the development of form and behaviour in amphibian embryos. Jour. Exp. Zool. 87: 245.

Elinson, R.P. 1977. Fertilization of immature frog eggs: cleavage and development following subsequent activation. J. Embryol. Exp. Morphol. 37(1):187-203.

Guyetant, R. 1970. Influence of grouping upon the growth and metamorphosis of the mid wife toad: Alytes obstetricans, evidence of a group effect. C.R. Hebd. Seances Acad. Sci. Ser. D. Sci. Natur. (Paris). 271(25): 2385-2388.

Herreid, C.F. and S. Kinney. 1967. Temperature and development of the woodfrog, Rana sylvatica, in Alaska. Ecology. 48: 579-590.

Inger, R.F. and J.P. Bacon. 1968. Annual reproduction and clutch size in Rain Forest Frogs from Sarawak. Copeia. 3: 602-606.

Ivanova, T.M. and L.I. Vinogradov. 1955. Specific significance of temperature on metamorphosis of Anuran larvae. C.R. Acad. Sci. URSS 102(6): 1223-1226.

*Izvanova, N.L. 1972. On the growth and development of Anura larvae under experimental conditions. Ekologiya. 4: 106.

†Knoepffler, L.P. 1962. Contribution a l'etude du genre Discoglossus (Amphibia, Anura). Vie et Milieu. 13: 1-94.

Koskela, P. and S. Pasanen. 1975. The reproductive biology of the female common frog, Rana temporaria L., in Northern Finland. Aquilo. Ser. Zool. 16: 1-22.

Kozlowska, M. 1971. Differences in the reproductive biology of mountain and lowland common frogs, Rana temporaria L. Acta. Biol. Cracov. Ser. Zool. 14: 17-32.

Kuramoto, M. 1975. Adaptive significance in oxygen consumption of frog embryos in relation to environmental temperatures. Comp. Biochem. Physiol. 52(1): 59-62.

Licht, L.E. 1971. Breeding habits and embryonic thermal requirements of the frogs, Rana aurora aurora and Rana pretiosa pretiosa in the Pacific North-West. Ecology. 52: 116-124.

Lovtrup, S. 1961. Morphological development and chemical differentiation during amphibian embryogenesis at low temperatures. Jour. Exp. Zool. 147: 227-232.

Lynn, W.G. and A. Edelman. 1936. Crowding and metamorphosis in the tadpoles. Ecology. 17: 104.

McLaren, I.A. and J.M. Cooley. 1972. Temperature adaptation of embryonic rate among frogs. Physiol. Zool. 45: 223-228.

Mondal, A.K. 1975. Frog breeding and its propagation in the context of frogleg industry in India. (Paper presented in the short term training course on

the improved techniques of frogleg processing and development of allied industries. Calcutta).

Moore, J.A..1939. Temperature tolerance and rates of development in the eggs of Amphibia. Ecology 20: 459-478.

Moore, J.A. 1942. The role of temperature in speciation of frogs. Biol. Symposia. 6: 189-213.

Olson, J.B. 1942. Changes in body proportions produced in frog embryos by supranormal temperatures. Proc. Soc. Exp. Biol. and Med. 51: 97.

Rugh, R. 1962. Experimental embryology. Techniques and procedures. Third edition. Minneapolis. Burgess Publishing Company.

Shvarts, S.S. and O.A. Pyastolova. 1970. Regulators of growth and development on amphibian. I. Specificity of effects. Ekologiya. 1: 58-62.

Somero, G.N. 1969. Enzymic mechanisms of temperature compensation: immediate and evolutionary effects of temperature on enzymes of aquatic poikilotherms. Am. Nat. 103: 517-530.

Woodruff, D.S. 1976. Embryonic mortality in Pseudophryne (Anura: Leptodactylidae). Copeia. 3: 445-449.

Van Gelder, J.J. and H.C.J. Oomen. 1970. Ecological observations on amphibia in the Netherlands. I. Rana arvalis Nilsson: reproduction, growth, migration and population fluctuations. Neth. J. Zool. 20: 238-252.

Zweifel, R.G. 1968. Reproductive biology of anurans of the arid southwest, with emphasis on adaptation of embryos to temperature. Bull. Am. Mus. Nat. Hist. 140: 1-64.

* Not consulted in original.

CONCLUDING REMARKS

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This thesis embodies the results of a detailed investigation on certain fundamental aspects of the Ecology and Development of Rana limnocharis Wiegmann found in Shillong and the neighbouring hills of the North-Eastern India which enjoys a sub-tropical climate. The fundamental aspects investigated are expected ultimately to have a direct or indirect bearing on the techniques to be evolved for the mass culture of this species. For over hundred years, anurans have been employed as material for classical experiments in academic institutions, while a few species have also been used as food items in Europe and other Western countries. The supply for the latter purpose still comes from nature and not from culture. Disturbance of the ecological balance due to fast urbanization and the indiscriminate use of insecticides have caused indirect threats to anuran populations all over the world by destruction of habitats and spawning sites. Such drastic landscape changes sadly affect their survival. While this may be true at the global level we do not have any data on frog census from the Indian sub-continent, though the census records of other countries do provide some basic information on these aspects. In England, the entire toad population had been known to be wiped out by automobiles as they cross roads, while on their spawning migrations. In Mississippi Valley, the use of DDT exterminated large portion of frog populations. There is, therefore, a great neccessity

to evolve successful frog culture techniques to augment the declining numbers in natural habitats.

Except for the few attempts mentioned before (Vide Supra) we have not yet evolved a sound technology on frog culture particularly in the Indian context. The first important aspect to be understood in this connection is the knowledge of the annual cycle and breeding habits of various species in a particular locality as the environmental conditions do vary from place to place. Intraspecific variations have also been reported to be dependent on local micro-environmental conditions as seen specially among high and low altitude populations of the same species. The second crucial requirement is the knowledge on the life cycle and preparation of developmental table for each species, which is indispensable for conducting experimental investigations to assess the mortality and survival rates and to evolve remedial measures. The knowledge of food and feeding habits of frogs forms another important aspect useful in culture practices. Feeding of frogs in confined environments has been found to be arduous task. Cemented ponds surrounded by (1 to 1.5 m) high fencing fitted with electric bulbs have been found to be conducive for maintaining frogs. These electric bulbs act as light traps and attract insects at night which are effectively used by frogs as food. Besides, the insects which form the high percentage of the food of a particular frog species can even be reared individually and supplied to them.

The study of the cytological changes in the pituitary glands and gonads reveals the sequential changes in the reproductive cycle in a given species of frog and based on this infommation the induced breeding can be attempted. The induced breeding technique as described in the present thesis has been proved to be very convenient and successful. The pituitary glands can be stored in absolute ethyl alcohol for many years with no loss of potency. At times, when specific hormones are not available, it becomes difficult to attempt induced breeding experiments. It is suggested that scientific laboratories or pharmaceutical firms could take up the preparation of vials of marketable size having specific dosage of the frog pituitary hormone. A fundamental knowledge of spawn size, fertility ratio, mortality and survival at early developmental stages and temperature tolerance is another important prerequisite for taking up the culture work. It is felt that the findings reported in this thesis provide adequate data to evolve in course of time a suitable culture technique for Rana limnocharis. By employing the induced breeding technique, the eggs can be grown up to the tadpole stage and then released in the ponds for further growth and maturity. While evolving a culture technique, low costs and economic considerations have to be kept in view. The findings reported in the present work can be profitably used for the culture of Rana limnocharis which will open up fresh avenues for further research to be undertaken in future.

ABOUT THE AUTHOR

Debjani Roy was born on September 13, 1953 at Barrackpore, West Bengal. She had her schooling at St. Mary's Convent, Ajmer, Rajasthan and passed the Higher Secondary Examination from the Rajasthan Board of Secondary Education in 1st Division in 1969. She secured 1st class at the M.Sc. examination in Zoology from Gauhati University, Gauhati in 1974; and since 1975 she has been working for the Ph.D. degree with Dr. M.K. Khare, Reader in Zoology, School of Life Sciences, NEHU, Shillong. She is a holder of Junior Research Fellowship awarded by the Council of Scientific and Industrial Research, New Delhi, and has published following papers.

1. Roy, D. and M.K. Khare. 1978. Normal Table of Development of Rana limnocharis Wiegmann. Proc. Nat. Acad. Sci. India 48(B): 5-16.

2. Roy, D. and M.K. Khare. 1979. Influence of embryonic limiting temperatures on the development of Rana limnocharis Wiegmann. Biol. J. Linn. Soc. 11(3): 279-288.

She has also presented papers at the annual sessions of the National Academy of Sciences India (1977), Indian Science Congress (1977) and Indian Society of Developmental Biologists (1977).