INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

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THE DEVELOPMENT & METAMORPHOSIS OF AN ENDANGERED FROG, *RANA LEPTOGLOSSA* (COPE, 1868)

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ABSTRACT
Rana leptoglossa has been listed as near-threatened amphibian species globally. But so far no attempt has been made to study the development and metamorphosis of *Rana leptoglossa* in its natural habitat or under captive conditions. Keeping in view the paucity of information as well as the endemic and endangered status of *R. leptoglossa*, studies were conducted on development and metamorphosis of the frog. The first division of the fertilized egg of *Rana leptoglossa* is completed within 90 minutes and the Morula (Gosner stage 8) is achieved after 9 hours of fertilization. The process of gastrulation (Gosner stage 10) started after 11 hours, neural plate (Gosner stage 13) formation took place after 15.30 hours and the neural fold (Gosner stage 14) was formed after 18 hours. The hatching of the embryo occurred after 4 days of fertilization. The gill buds (Gosner stage 19) appeared after 7 days. The hind limbs (Gosner stage 26) appeared after 26-27 days, and were fully developed (Gosner stage 40) after 55-58 days of fertilization. The forelimb buds (Gosner stage 42) appeared after 61-63 days when the tadpole was found to have the maximum length (46-47 mm). Thereafter, the degeneration of the tail of the tadpole started and the metamorphosis was completed in 68-72 days when a tadpole was metamorphosed in to a froglet (Gosner stage 46). Comparatively long duration of metamorphosis of *R. leptoglossa* might be due to the prevailing condition of temperature and daylength as well as genetic programming. The present study seems to be first of its kind in which various stages of development and metamorphosis of the endangered frog, *Rana leptoglossa* have been established.

KEY WORDS: Ranidae, Gosner stage, Development, Metamorphosis, Embryology, *Rana leptoglossa*.

INTRODUCTION

In India 303 species of amphibians were listed but studies of developmental stages and metamorphosis of frogs were on few species: *Bufo melanostictus* (Khan, 1965); *Rhacophorus malabaricus* (Sekar, 1989); *Rana limnocharis* (Roy, 1990); *Polypedates maculates* (Misra and Das, 1984; Kanamadi and Jirrakali, 1992); *Hyla annectans* (Ao and Bordoloi, 2001); *Chrixalus simus* (Deuti, 2001); *Philautus glandulosus* (Biju, 2003); *Philautus leucorhinus* (Gururaja *et al*., 2005); *Polypedates lecomystax* (Iangrai, 2007); *Rhacophorus bipunctatus* (Iangrai, 2007) and *Rhacophorus lateralis* (Biju, 2009). Studies were also conducted on external morphology, bucco-pharyngeal anatomy and development rate of the tadpoles of two Asian Ranidae (Amphibia: Anura), *Hylarana humeralis* (Boulenger, 1887) and *Hylarana leptoglossa* (Cope, 1868) (Bortamuli *et al*., 2010).

The growth and development rates in anurans are influenced by numerous environmental factors such as temperature (Kaplan, 1980; Saidapur and Hoque, 1995), rainfall (Lynch and Wilczynski, 2005), photoperiod (Saidapur, 1989), pool desiccation (Lind *et al*., 2008), food supply and diet quality (Berven and Chandra, 1988; Nicieza *et al*., 2006), environmental iodine levels (Dodd and Dodd, 1976), pond hydrology (Ryan and Winne, 2001), and breeding habitat (Kaplan, 1980; Hayes, 1997). The growth and development of the anurans are also influenced by intrinsic factors such as tadpole size or egg size and yolk reservoirs (Duellman and Trueb, 1994). The combination of these factors ultimately influences the time taken to program from eggs to a froglet (Morrison and Hero, 2003). The anuran metamorphosis is controlled by the hypothalamo-hypophyseal-thyroid axis involving actions of several hormones (Huang and Brown, 2000; Furlow and Neff, 2006; Page *et al*., 2008). Environmental factors stimulate release of thyrotropin releasing hormone (TRH) by the hypothalamus, which stimulates secretion of thyroid stimulating hormone (TSH) from the pituitary. TSH stimulates secretion of thyroid hormones (TH) namely 3, 5, 3́-triiodothyronine (T_3) and 3, 5, 3́, 5́-tetraiodothyronine (T_4) from the thyroid gland. An increased concentration of T_4 has been reported to accelerate metamorphosis of anuran tadpoles (Page *et al*., 2008). Besides thyroid hormones, prolactin also plays a critical role in regulation of anuran larval development and metamorphosis (Dodd and Dodd, 1976; Takada and Kasai, 2003). Prolactin (PRL) is widely considered to be the juvenile hormone of tadpoles and counteracts the stimulatory effects of thyroid hormones on metamorphosis (Takada and Kasai, 2003).

Anuran larval development is divided into three specific periods, i.e., pre-metamorphosis, pro-metamorphosis and metamorphosis climax. Pre-metamorphosis refers to a period when embryogenesis and early tadpole growth and development take place in the absence of thyroid hormones or very less thyroid hormone. During pro-metamorphosis, hind limbs undergo morphogenesis as exemplified by the differentiation of the toes and rapid extensive growth of hind limbs. This period is

characterized by rising concentration of endogenous thyroid hormones (Rojas *et al.,* 2003). The metamorphic climax is the period of radical changes that culminate in the loss of most larval characters with rapid differentiation in tadpole marked by the initiation of tail regression, complete resorption of the tail, and development of structures and functions *de novo* that are essential to the adult due to high thyroid hormones (Hall and Larson, 1998; Mc Diarmid and Altig, 2000).

So far no attempt has been made to study the development and metamorphosis of the endangered frog, *Rana leptoglossa*. Therefore, it was thought worthwhile to investigate the developmental stages and metamorphosis of the frog, *Rana leptoglossa*. Based on the present findings, it has been estimated that the development and metamorphosis of *Rana leptoglossa* is completed within 68-72 days.

MATERIALS AND METHODS

In order to study the development and metamorphosis of *Rana leptoglossa*, observations were made on the development and metamorphosis during three consecutive breeding periods in the years 2005, 2006 and 2007. Fresh spawns were collected from the breeding sites, brought to the laboratory and maintained in rectangular plastic trays to allow further development and metamorphosis. The water of the plastic trays containing the developing tadpoles was changed regularly with the pond water. The tadpoles were feed with phytoplankton, zooplankton and minced earthworms *ad libitum*. Different developmental stages of the frog (i.e., from fertilized eggs to metamorphosed froglets) were preserved in 4% formaldehyde solution. External morphology and measurements (in mm) were recorded from well preserved specimens with the help of a Vernier caliper. During the study period, the developmental stages of *Rana leptoglossa* were studied from the time of spawning and fertilization (0 Gosner stage, 0 h), till metamorphosis of the tadpoles into froglets under both natural and captive conditions. Early developments stages were collected at an interval of 15 minutes up to 48 hours to find out the embryogenesis of developing eggs, which were observed under the binocular microscope attached with photographic facilities (Zeiss Stemi 2000C Binocular Microscope with KL 1500 LCD camera).

Staging of the tadpoles were performed as per the system proposed by Gosner (1960). The photographs of the early developmental stages were taken from the preserved specimens and presented in Plates: 1, 2, 3, 4 & 5, while the photographs of the live specimens are presented in Plate 6.

RESULTS

The important larval stages were differentiated on the basis of age, size and external morphological characters. Various stages of development and metamorphosis of *Rana leptoglossa* were divided into 17 major sub-headings with 46 Gosner stages (1960). A brief account of the sub-headings and each Gosner Stage has been given in the following sections:

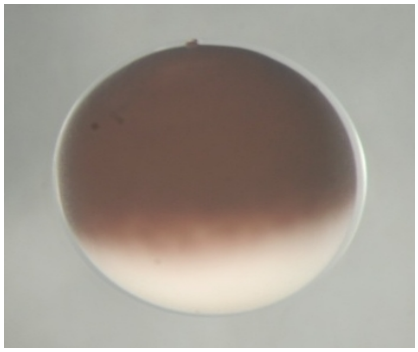

Fig. 1.1: Fertilized egg
(Gosner Stage 1)

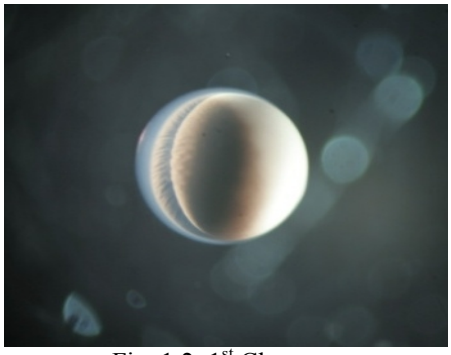

Fig. 1.2: 1st Cleavage
(Gosner Stage 2)

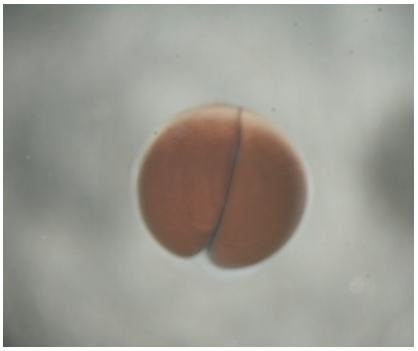

Fig. 1.3: Two cell
(Gosner Stage 3)

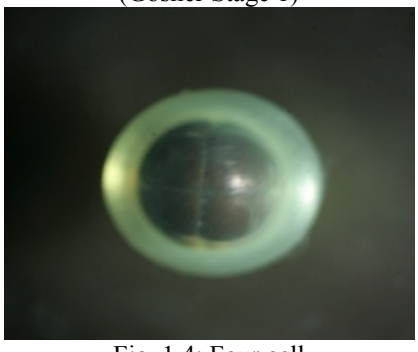

Fig. 1.4: Four cell
(Gosner Stage 4)

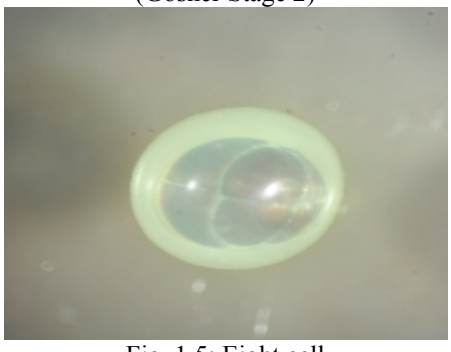

Fig. 1.5: Eight cell
(Gosner Stage 5)

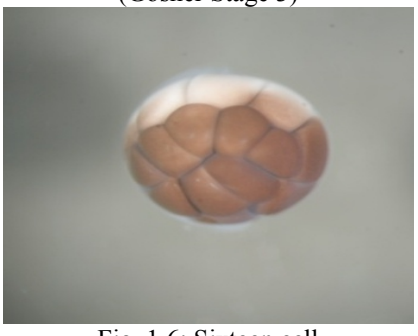

Fig. 1.6: Sixteen cell
(Gosner Stage 6)

PLATE 1: Developmental stages (Gosner stages 1 – 6) of *Rana leptoglossa*

I. Fertilized eggs:

Gosner Stage 1: Fertilized egg (Age 0 hr, Diameter 0.5 mm) - The eggs were black in colour, spherical in shape

and measured about 0.5 mm in diameter. The animal pole was uppermost, pigmented dark brown, and vegetal pole was lowermost, white in colour, easy to distinguish under the binocular microscope (Zeiss Stemi 2000C) (Fig. 1.1).

Gosner Stage 2: One cell stage (Age 1.00 hr, Diameter 0.6 mm) - A lightly pigmented area (gray crescent) appeared between the animal pole and vegetal pole towards the pigmented hemisphere (Fig. 1.2).

II. Cleavage stages:

Gosner Stage 3: Two cell stage (Age 1.30 hrs, Diameter 0.8 mm) - The meridional cleavage furrow originating at the animal pole proceeded to the vegetal pole and gradually divided the fertilized egg completely into two equal blastomeres (Fig. 1.3).

Gosner Stage 4: Four cell stage (Age 2.00 hrs, Diameter 1.0 mm) - The second meridional furrow, which started at the animal pole, extended to the vegetal pole at right angle to the first furrow. Altogether there were four blastomeres (Fig. 1.4).

Gosner Stage 5: Eight cell stage (Age 2.30 hrs, Diameter 1.1 mm) - The third cleavage was latitudinal, slightly above the equator, which formed eight blastomeres. The four smaller micromeres of the animal pole were pigmented dark brown, where as the four bigger macromeres of the vegetal pole were unpigmented (Fig. 1.5).

Gosner Stage 6: Sixteen cell stage (Age 3.00 hrs, Diameter 1.2 mm) - The cleavage furrows were vertical, one passed through pigmented micromeres and another through unpigmented macromeres resulting in 16 cells altogether (Fig. 1.6).

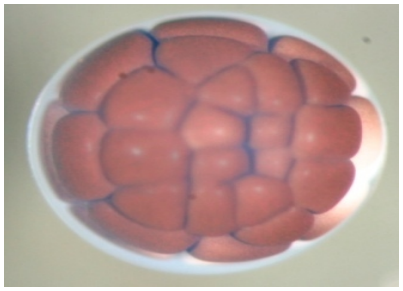

Fig. 1.7: 32-Cell
(Gosner Stage 7)

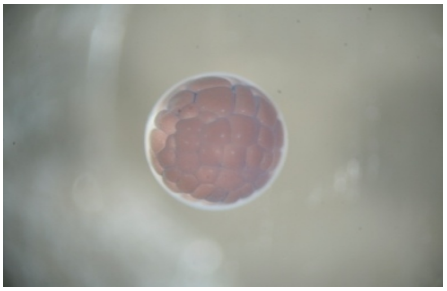

Fig. 1.8: 64-Cell
(Gosner Stage 8)

Fig. 1.9: 128-Cell
(Gosner Stage 9)

PLATE 1: Developmental stages (Gosner stages 6 – 9) of *Rana leptoglossa*

Gosner Stage 7: Thirty-two cell stage (Age 3.30 hrs, Diameter 1.3 mm) - The latitudinal cleavage furrows of the micromeres and macromeres resulted in formation of 16 micromeres and 16 macromeres resulting in 32 cells in total (Fig. 1.7).

Gosner Stage 8: Mid-cleavage/Morula (Age 9.00 hrs, Diameter 1.4 mm) - As a result of further cleavage/cell division, the developing embryo attained the stage of morula (a collection of 64 to 128 cells) (Figs.1.8 & 1.9).

Gosner Stage 9: Late cleavage/blastula (Age 10.30 hrs, Diameter 1.5 mm) - Due to repeated cell divisions, the fertilized eggs attained late blastula stage. The pigmented region extended over the vegetal pole, which marked the beginning of the epibolic movement of the micromeres onto the macromeres (Fig. 1.10).

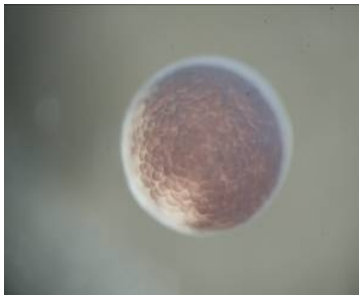

Fig. 1.10: Late blastula
(Gosner Stage 10)

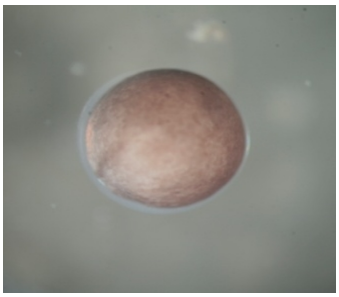

Fig. 1.11: Blastopore
(Gosner Stage 11)

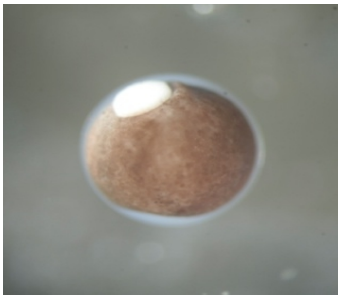

Fig. 1.12: Yolk Plug
(Gosner Stage 12)

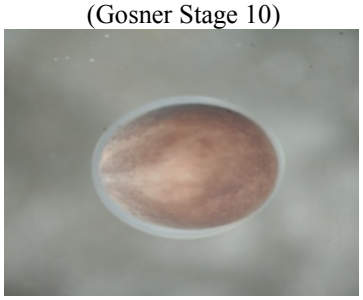

Fig. 1.13: Neural plate
(Gosner Stage 13)

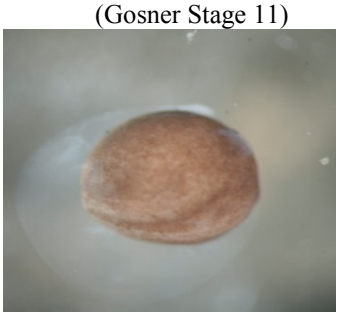

Fig. 1.14: Neural groove
(Gosner Stage 15)

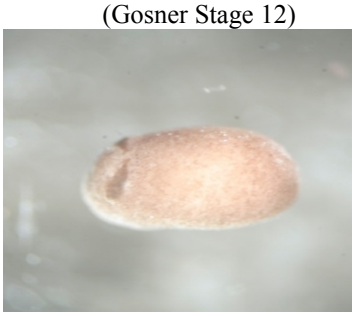

Fig. 1.15: Eye cup
(Gosner Stage 17)

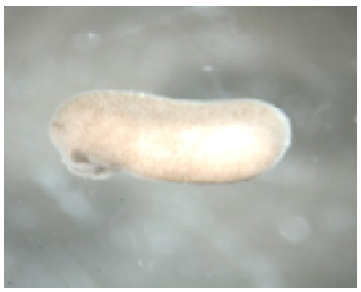

Fig. 1.16: Mid tail bud (Gosner Stage18)

Fig. 1.17: Late tail bud (Gosner Stage19)

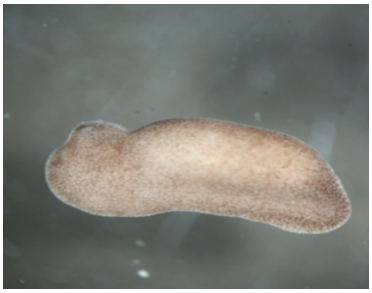

Fig. 1.18: Tail formation (Gosner Stage 20)

PLATE 2: Developmental stages (Gosner stages 10 – 20) of *Rana leptoglossa*.

III. Gastrulation stages:

Gosner Stage 10: Crescent-shape dorsal lip (11.00 hrs, Diameter 1.7 mm) - The developing blastula underwent gastrulation. It elongated and rotated and measured about 1.5 to 2 mm in length. Appearance of crescent shaped dorsal lip due to involution of the micromeres indicated the beginning of gastrulation. The unpigmented zone of the vegetal hemisphere was reduced due to continued migration of the pigmented micromeres towards the vegetal pole (Fig. 1.11).

Gosner Stage 11: Horse-shoe shaped dorsal lip (11.30 hrs, Diameter 1.9 mm) - The epibolic migration of micromeres over the vegetal pole reduced the exposed area of unpigmented macromere which was surrounded by the lateral lips of the semicircular or horse-shoe shaped blastopore (Fig. 1.11).

Gosner Stage 12: Development of yolk plug (12.30 hrs, Diameter 2.1 mm) - A well developed yolk plug appeared. The ventral lip of blastopore shifted to the posterior end. The uninvaginated macromeres, surrounded by the blastoporal lips, protruded a little and constituted the yolk plug (Fig. 1.12).

IV. Neuralation stages:

Gosner Stage 13: Neural plate (15.30 hrs, Length 2.3 mm) - The developing embryo became slightly elongated. The dorsal surface was flattened to form the neural plate, which was differentiated with the concentration of pigments along its borders (Fig. 1.13).

Gosner Stage 14: Neural folds (18.00 hrs, Length 2.6 mm) - The neural fold became distinct with broad cerebral and narrow spinal cord regions of the neural plate. The neural folds gradually approached each other from blastopore to anterior region (Fig. 1.13).

Gosner Stage 15: Elongation and rotation (Neural groove) (20.00 hrs, Length 2.8 mm) - The posterior end of the embryo became elongated. The neural folds came closer and touched each other in both cerebral and spinal cord regions, forming a shallow neural groove which was broader in the cerebral region (Fig. 1.14).

Gosner Stage 16: Neural tube (Age 3 days, Length 3.0 mm) - The neural folds had fused completely to form the neural tube, which was raised at the mid-dorsal ridge and demarcated by a darkly pigmented strand (Fig. 1.14).

V. Early tail bud stages:

Gosner Stage 17: Tail bud stage (Age 4 days, Length 3.5 mm) - On the 4th day, the developing embryos hatched into hatchlings/tadpoles. It measures 2 to 3.5 mm in length. Tail bud appeared at the posterior end of the embryo. It was wider than long, directed dorso-posteriorly and marked off from the body by a ventral notch (Fig. 1.15).

Gosner Stage 18: Muscular response stage/olfactory pits (Age 5 days, Length 3.5-4.5 mm) - The head region was well developed with optic bulges and bulges of the gill plates. Oral suckers were indicated by two heavily pigmented elongated areas joined medially by a narrow lightly pigmented band below the stomodeum. The stomodeal depression was seen between the oral suckers. Due to the gradual elongation of the embryo, the tail started curving laterally to right or left, within the contour of the vitelline membrane. There was still gradual elongation of the embryo and the tail started curving laterally to the left (Fig. 1.16).

VI. Mid tail bud stage:

Gosner Stage 19: Gill buds stage (Age 7 days, Length 4.5-5.0 mm) - The developing tadpole completely differentiated into head, abdomen and tail. External gill buds became prominent (Fig. 1.17).

VII. Late tail bud stage:

Gosner Stage 20: Gill circulation and tail elongation stage (Age 9 days, Length 5.0-6.5 mm) - The tail elongated, gill buds appeared and mouth opened. Gills distinct, rudimentary branching at distal end and oral suckers nipple-shaped (Fig. 1.18).

VIII. External gills stages:

***Gosner Stage 21*:** Secondary hatching of larva (Age 11 days, 6.5-8.1 mm) - Well developed branched gills were seen in the developing tadpole. Body musculatures developed. Tail fins and cornea became transparent. Gills and fins circulation started (Fig. 1.19).

***Gosner Stage 22*:** Tail fin circulation stage (Age 13 days, Length 8.2-9.3 mm) - Tail fin circulation started at the base of anterior part of dorsal fin, just above the trunk. Tail fins were transparent. Mouth was slightly wider (Fig. 1.20).

IX. Operculum, oral disc and pigmentation stages:

***Gosner Stage 23*:** Opercular fold development stage (Age 15 days, Length 9.4-10.5 mm) - Operculum covered bases of external gills. Jaws were not keratinized. Upper and lower labial fringes developed papillae and faint labial ridges. Pigmentation on tail began, cloaca not opened (Fig. 1.21).

Gosner Stage 24: Opercular fold closed on right side (Age 17 days; Length 10.5 -11.5 mm) - Operculum closed on right side. Oral disc was well developed and pigmentation started. The developing tadpoles were black in colour (Fig. 1.22).

X. Feeding stage:

Fig. 1.19: Ventral mouth (Gosner Stage 21)

Fig. 1.20: Tail fin circulation (Gosner Stage 22)

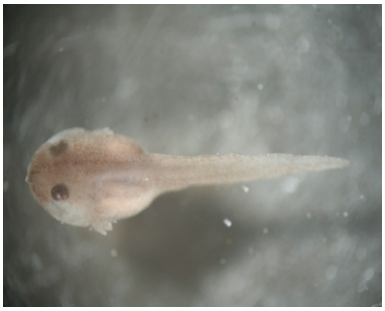

Fig. 1.21: External gills (Gosner Stage 23)

Fig 1.22: External gills atrophy (Gosner Stage 24)

Fig. 1.23: Operculum closes (Gosner Stage 25)

Fig. 1.24: Spiracle forms on left (Gosner Stage 26)

PLATE 3. Developmental stages (Gosner stages 21 - 26) of *Rana leptoglossa***.**

Gosner Stage 25: Operculum of embryo closed on left side (Age 18-25 days, Length 11.6 – 21.5 mm) - External gills disappeared, operculum closed on left, and spiracle formed on left. The tail lightly pigmented, the anal tube opened and the tadpole was found to be a voracious feeder (Fig. 1.23).

XI. Hind limb bud development stages:

Gosner Stage 26: Length of limb bud less than half of its diameter (Age 26-27days, Length 21.5-23.5 mm) - The tail increased in length. Hind limb bud appeared at the junction of tail and trunk, and was less than half of its diameter. Pigmentation spreaded dorsal to anal fins (Fig. 1.24).

Gosner Stage 27**:** Length of limb bud equal to half of its diameter (Age 28-29 days, Length 23.5-25.0 mm) - Length of the hind limb bud was equal to half of its diameter. The patches of pigmentation in the tail fin spreaded considerably.

Gosner Stage 28**:** Length of limb bud equal to its diameter (Age 30-31 days, Length 25.1- 26.0 mm) - Distal end of the hind limb bud was slightly conical. The length of limb bud was equal to its diameter.

Gosner Stage 29**:** Length of limb bud was equal to one and half times its diameter (Age 32-33 days, Length 26.1-28.0 mm) - Distal half of conical hind limb was equal to one and half times its diameter.

Gosner Stage 30: Length of limb bud was equal to twice of the diameter (Age 34-35 days, Length 28.1-29.0 mm) - Distal end of hind limb bud was equal to twice of the diameter and slightly bent ventrally. No pigmentation on limb bud.

Fig. 1.25: Hind limbs and toe differentiation (Gosner Stage 31- 39)

Fig. 1.26: Appearance of fore limbs bud (Gosner Stage 40-42)

Fig. 1.27: Emergence of both forelimbs and hind limbs (Gosner Stage 42)

PLATE 4: Developmental stages (Gosner stages 31 - 42) of *Rana leptoglossa*.

XII. Toe differentiation and development stages:

Gosner Stage 31: Foot paddle stage (Age 36-40 days, Length 29.2-30.0 mm) - The developing tadpoles possessed well developed hind limbs and differentiated pentadactyle toes. Hind limb comprised toe pads and webs. Spiracle opening was on the left side of the head in the form of a small tube. Toe differentiation occurred during Gosner stages 31 to 39 (Fig. 1.25).

Gosner Stage 32: First indentation (Age 41 days, Length 31.0 mm) - The head and trunk were well developed. The margin of the foot-paddle became slightly indented on the dorsal side which marked the prominences of the future 4th and 5th toes.

Gosner Stage 33: Second indentation (Age 42 days, Length 32.0 mm) - The margin of the foot-paddle became indented on the ventral side behind the prominence of 4th toe, and marked the prominence of the 3rd, 4th and 5th toes.

Gosner Stage 34: Third indentation: (Age 44 days, Length 33.0 mm) - The margin of foot paddle became indented, on the ventral side behind the prominence of 3rd toe, which marked the prominence of 2nd, 3rd, 4th and 5th toes.

Gosner Stage 35: Fourth indentation (Age 45 days, Length 34.0 mm) - The margin of the foot paddle was indented behind the 2nd toe demarcating the prominence of the 1st toe. All the five toes were visible and separated from each other.

Gosner Stage 36: Margin of the 5th toe web directed towards the tip of 2nd toe (Age 46-47 days, Length 34.1-37.0 mm) - The margin of the 5th toe web was directed towards the tip of the 2nd toe.

Gosner Stage 37: Margin of 5th toe web directed towards the tip of 1st toe (Age 48-50 days, Length 37.1- 39.0 mm) - The margin of 5th toe web was directed towards the tip of 1st toe. Pigmentation appeared in the 4th and 5th toes along the foot. Toes were longer and all toes were separated.

Gosner Stage 38: Appearance of metatarsal tubercle (Age 51-52 days, Length 39.1- 40.0 mm) - The inner metatarsal tubercle became a small outgrowth. Pigmentation appeared in 3rd, 4th and 5th toe along the foot.

Gosner Stage 39: Appearance of sub-articular tubercles in the toes (Age 53-54 days, Length 40.1-43 mm) - The sub-articular tubercles appeared on the inner surface of the toes as light patches. The inner metatarsal tubercle became a small oval outgrowth.

XIII. Well developed hind limb stages:

Gosner Stage 40: Toe pads complete (Age 55-58 days, Length 43.1- 44.5 mm) - Hind limbs were well developed with differentiated toes. Mouthparts atrophied, vent tube and forelimb buds visible. The cloacal tail piece was not reduced.

Gosner Stage 41: Cloacal tail piece reduced (Age 59-60 days, Length 44.6- 46.0 mm) - The tail reabsorption started from this stage. The cloacal tail piece gets reduced and only a narrow strip remained over and in between bases of the thigh as tail stub. Mouth parts atrophied and vent tube disappeared completely (Fig. 1.26).

XIV. Eruption of fore limb stage:

Gosner Stage 42: Forelimbs emerge (Age 61-63 days, Length 46.1-47.0 mm) - The emergence of both forelimbs took place. Left forelimb emerged through spiracle opening and right forelimb emerged by rupturing opercular fold. Mouth restructuring took place anterior to nostril. This was the stage which represented the maximum length of tadpole (Fig. 1.27).

XV. Tail resorption and mouth restructuring stages:

Gosner Stage 43: Angle of mouth between eye and nostril (Age 64-66 days, Length 36 mm) - The widening angle of mouth had reached a point midway between nostril and the anterior margin of the eye. The tail resorption started, the dorsal and ventral fins started shrinking (Fig. 1.28).

Gosner Stage 44: Angle of mouth reached the middle and beneath of the eye and tail greatly reduced (Age 67-68 days, Length 22 mm) - After the completion of fore limbs and hind limbs, tail resorption was very fast. Tadpoles started jumping. The widening angle of mouth had reached the level of the middle of the eye and beneath the eye. The dorsal and ventral fins had disappeared. The tail was

reabsorbed considerably, and was as long as the femur (Fig. 1.29).

Fig. 1.28: Hind limb with five toes
(Gosner Stage 31- 39)

Fig. 1.29: Fore limb with four fingers
(Gosner Stage 40 – 42)

PLATE 5. Limbs (Gosner stages 26 - 42) of *Rana leptoglossa*

XVI. Metamorphosed tadpole stage:

Gosner Stage 45: Angle of the mouth reached posterior margin of the eye (Age 69-71 days, Length 17 mm) - The widening of the mouth reached the posterior margin of the eye. Tadpoles come out from the water. Once they came out of water, they changed their colour from black to brown. The tail reabsorption was complete but still remained a triangular stub. A deep brown rounded tail stump at the base of the cloaca was visible (Fig. 1.30).

XVII. Metamorphosed froglet stage:

Gosner Stage 46: Complete metamorphosed froglet (72 days, Length 16.5 mm) - Hind limbs and forelimbs were well developed, re-absorption of tail was complete and the tail stub disappeared completely. The metamorphosis was complete, and a juvenile froglet was formed (Fig. 1.31).

Fig. 1.30: Froglet with tail stub
(Gosner Stage 44; 66 days)

Fig. 1.31: Young froglet
(Gosner Stage 45; 68 days)

Fig. 1.32: Metamorphosed froglet,
(Gosner Stage 46; 70 days)

Fig. 1.33: A juvenile *R. leptoglossa*
(72days)

PLATE 6. Developmental stages (Gosner stages 44 - 46) of *Rana leptoglossa*

DISCUSSION

Findings of the present study indicate that the first division of the fertilized egg of *Rana leptoglossa* is completed within 90 minutes and the Morula (Gosner stage 8) is achieved after 9 hours. The process of gastrulation (Gosner stage 10) started after 11 hours, neural plate (Gosner stage 13) formation takes place after 15.30 hours and the neural fold (Gosner stage 14) was formed after 18 hours. The hatching of the embryo occurred after 4 days of fertilization. The gill buds (Gosner stage 19) appeared after 7 days. The hind limbs (Gosner stage 26) appeared after 26-27 days, and were fully developed (Gosner stage 40) after 55-58 days of fertilization. The forelimb buds (Gosner stage 42) appeared after 61-63 days when the tadpole was found to have the maximum length (46-47 mm). Thereafter, the degeneration of the tail of the tadpole started after 63 days and the metamorphosis was completed in 68-72 days when a tadpole was metamorphosed in to a froglet (Gosner stage 46). The prevailing climatic conditions during metamorphosis of *Rana leptoglossa* were as follows: optimum temperature $25.52^0C \pm 0.43^0C$ to $29.83^0C \pm 0.23^0C$, daylength 12.71 h ± 0.03 h to 13.16 h ± 0.04 h and relative humidity 75.83% ± 1.19% to 80.67% ± 0.97%. Comparatively long duration of metamorphosis of *R. leptoglossa* might be due to the prevailing conditions of temperature and daylength.

In India the duration of development and metamorphosis of anurans has been found to vary from species to species. For example, the metamorphosis is reportedly completed in 94 days in *Rana cyanophlyctis*, 68 days in *Rhacophorus malabaricus*, 64 days in *Hyla annectans*, 60-61 days in *Polypedates leucomystax*, 59-60 days in *Rhacophorus bipunctatus*, 55 days in *Polypedates maculates*, 35-50 days in *Bufo melanostictus*, 28 days in *Philautus glandulosus* and 19 days in *Philautus leucorhinus*.

Based on the present findings, it can be concluded that the development and metamorphosis of *Rana leptoglossa* is completed within 68-72 days during the month of April to August when climatic factors were favorable. The present study seems to be the first of its kind in which various stages of development and metamorphosis of the endangered frog, *Rana leptoglossa* and its life cycle have been established (Plate 7). These findings can be used in planning the conservation of the frog under its natural habitats and its breeding under captive conditions.

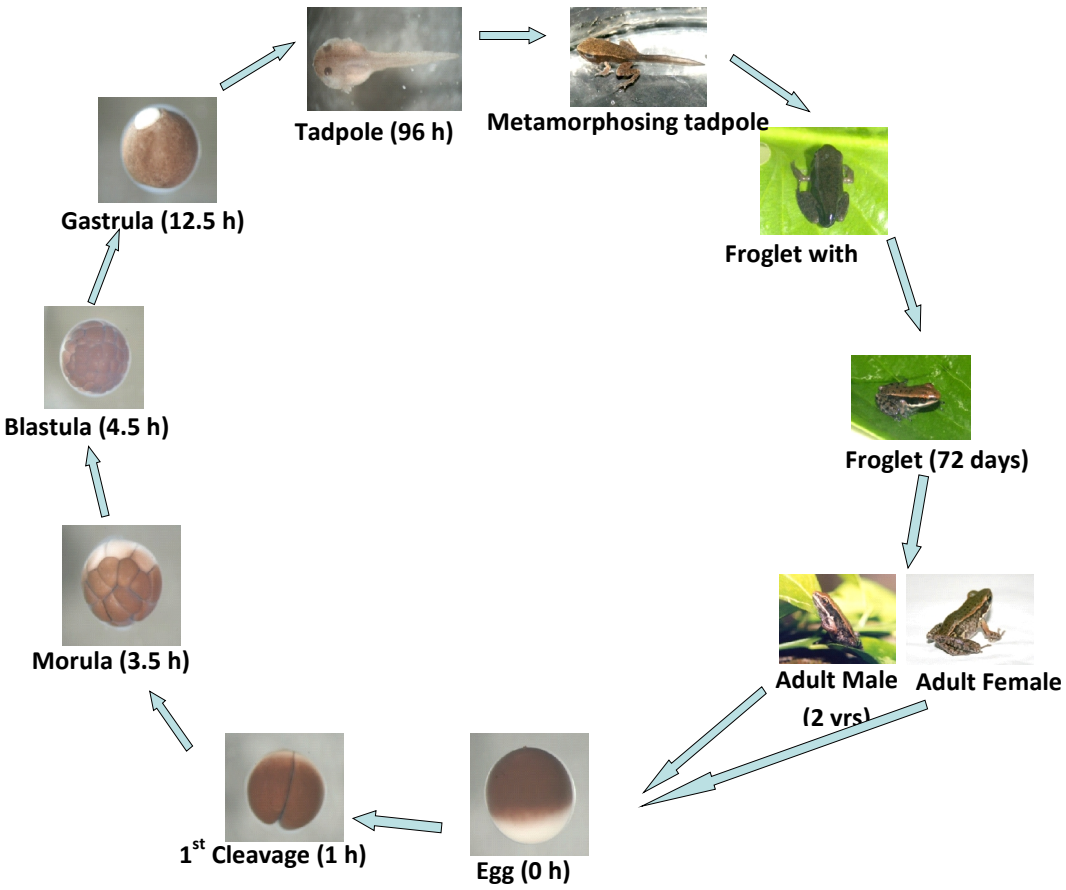

PLATE 7. Life cycle of *Rana leptoglossa*

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