CHAPTER - 3

Studies on developmental stages and metamorphosis of the frog, *Rana leptoglossa*

Introduction

Amphibian development has been investigated extensively by many embryologists (Pedersen and Schatten, 1998). One of the prominent life history characteristics common to most living amphibians is the presence of an aquatic larval period, which immediately follows the initial embryonic development after fertilization and ends with the completion of metamorphosis (Diwan and Dhakad, 1996; Bishop *et al*., 2006). Metamorphosis is a post-embryonic period of profound morphological changes by which the animal alters its mode of living from aquatic gill breathing tadpole to air-breathing terrestrial adult (Ronald *et al*., 2000; Shi, 2000). Metamorphosis and developmental stages in anurans exhibit a higher degree of modifications and specialization in comparison to apodans and urodelans (McDiarmind and Altig, 2000). Metamorphosis has been found to be a series of transcriptional programs controlled by thyroid hormones which result in reorganization of most tissues and organs of the tadpole (Galton, 1983).

During metamorphosis, distinct remodeling has been reported in tail resorption (Huang and Brown, 2000; Yaita and Nakajina, 1997), muscles (Nicolas *et al*., 1998; Gaillard *et al*., 1999; Cai *et al*., 2007), intestine (Shi and Brown, 1993), pancreas (Shi and Brown, 1990), kidney (pronephros to metanephros), respiratory organs (gills to lungs) (Dodd and Dodd, 1976), liver (Atkinson *et al*., 1998), nose
(Higgs and Burd, 2001), pituitary gland (Kikuyama et al., 1993; Huang et al., 2001), and most of the skeleton (Trueb and Hanken, 1992).

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). These factors act in synergy to increase or decrease the growth and development rates occurring within and between each life stage in anurans. The combination of these effects 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; Furrow 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, 3′-triiodothyronine (T₃) and 3, 5, 3′, 5′-tetraiodothyronine (T₄) from the thyroid gland. An increased concentration of T₄ has been reported to accelerate metamorphosis of anuran tadpoles (Page et al., 2008). In Xenopus laevis, corticotrophin releasing hormone instead of thyrotropin releasing hormone has been reported induce thyroid
stimulating hormone (TSH) secretion from pituitary at the onset of metamorphosis (Boorse and Denver, 2004).

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). Prolactin mainly helps in metamorphosis in early part of the life history. It has not been detected after 34th day of developing tadpole in gray tree frog, *Hyla versicolor* (Beachy et al., 1999). The growth of post-metamorphic anurans is reportedly stimulated by somatotropin but not by prolactin (Frye et al., 2004).

Anuran larval development is divided into three specific periods, i.e., pre-metamorphosis, pro-metamorphosis and metamorphosis climax (Misra and Dash, 1984 a, b). After hatching from the eggs, anurans start life as free swimming larvae with little growth and much differentiation. 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).

Gosner (1960) gave a simplified table for staging anuran embryos and larvae with notes of identification. Mc Diarmid and Altig (2000) suggested the complete tables of development for accurate comparison of development stages in different anurans with 46 Gosner stages. In India, out of 303 species of amphibians (www.amphibianweb.org, 20 December, 2010), the developmental stages of only 12-14 species have been studied and documented (Das and Dutta, 2007). The total duration of metamorphosis of anurans varies from species to species, such as in case of Bufo melanostictus: 35-50 days (Khan, 1965), Rana cyanophlyctis: 94 days (Mohanty-Hejmadi and Dutta, 1986), Polypedates maculates: 55 days (Mohanty et al., 1997), Rhacophorus malabaricus: 68 days (Sekar, 1989), Hyla annectans: 64 days (Ao and Bordoilo, 2001), Philautus glandulosus: 28 days (Biju, 2003), Ramanella montane: 160 days (Krishna et al., 2004), Polypedates leucomystax: 60-61 days (Iangrai, 2007), and Rhacophorous bipunctatus: 59-60 days (Iangrai, 2007).

So far no attempt has been made to study the development and metamorphosis of the endangered frog, Rana leptoglossa. A sound knowledge of its developmental stages and metamorphosis will be of immense help in establishing the breeding biology of the rare frog as well as in its conservation. Therefore, it was thought worthwhile to investigate the developmental stages and metamorphosis of Rana leptoglossa.

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. Air temperature, daylength, rainfall and relative humidity at the breeding sites were recorded (please see Chapter 1: Tables 1.4, 1.5 & 1.6). Water temperatures and water pH of the breeding sites were also recorded with ordinary thermometer and pH indicator paper (Universal indicator pH 1-10, MERCK), respectively (please see Chapter 2: Table 2.4). 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 embryos 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 was 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: 3.1, 3.2, 3.3, 3.4 & 3.5, while the photographs of the live specimens are presented in Plate 3.6.

**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:

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. 3.1).

**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. 3.2).

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. 3.3).

**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. 3.4).
**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, whereas the four bigger macromeres of the vegetal pole were unpigmented (Fig. 3.5).

**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. 3.6).

**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. 3.7).

**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. 3.8 & 3.9).

**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. 3.10).

### 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. 3.11).
**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. 3.11).

**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. 3.12).

**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. 3.13).

**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. 3.13).

**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. 3.14).

**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. 3.14).
V. Early tail bud stages:

**Gosner Stage 17**: Tail bud stage (Age 4 days, Length 3.5 mm) - On the 4\textsuperscript{th} 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. 3.15).

**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. 3.16).

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. 3.17).

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. 3.18).

**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. 3.19).

**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. 3.20).

**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. 3.21).

**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. 3.22).

**X. Feeding stage:**

**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. 3.23).
XI. Hind limb bud development stages:

_Gosner Stage 26:_ Length of limb bud less than half of its diameter (Age 26-27 days, 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. 3.24).

_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.

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. 3.25).
**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 4\textsuperscript{th} and 5\textsuperscript{th} 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 4\textsuperscript{th} toe, and marked the prominence of the 3\textsuperscript{rd}, 4\textsuperscript{th} and 5\textsuperscript{th} 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 3\textsuperscript{rd} toe, which marked the prominence of 2\textsuperscript{nd}, 3\textsuperscript{rd}, 4\textsuperscript{th} and 5\textsuperscript{th} toes.

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

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

**Gosner Stage 37**: Margin of 5\textsuperscript{th} toe web directed towards the tip of 1\textsuperscript{st} toe (Age 48-50 days, Length 37.1- 39.0 mm) - The margin of 5\textsuperscript{th} toe web was directed towards the tip of 1\textsuperscript{st} toe. Pigmentation appeared in the 4\textsuperscript{th} and 5\textsuperscript{th} 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 3\textsuperscript{rd}, 4\textsuperscript{th} and 5\textsuperscript{th} 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. 3.26).

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. 3.27).

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. 3.28).

_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. 3.29).

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. 3.30).

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. 3.31).

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 into a froglet (Gosner stage 46). The prevailing climatic conditions during metamorphosis of *Rana leptoglossa* were as follows: optimum temperature 25.52\(^{\circ}\)C ± 0.43\(^{\circ}\)C to 29.83\(^{\circ}\)C ± 0.23\(^{\circ}\)C, 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. Low availability of iodine at K. R. F (endemic goiter region) may also be responsible for slow rate of metamorphosis.

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* (Mohanty-Hejmadi and Dutta, 1979), 68 days in *Rhacophorus malabaricus* (Sekar, 1989), 64 days in *Hyla annectans* (Ao and Bordoiloi, 2001), 60-61 days in *Polypedates leucomystax* (Iangrai, 2007), 59-60 days in *Rhacophorus bipunctatus* (Iangrai, 2007), 55 days in *Polypedates maculates* (Mohanty and Dutta 1986) and 35-50 days in *Bufo melanostictus* (Khan, 1965). The *Philautus* is the only genus of Western Ghats in India which directly develops into a froglet. The development of this species occurs within the egg membranes, and there are no free swimming tadpole stages. The total duration of its development is
completed in 28 days in *Philautus glandulosus* (Biju, 2003) and only in 19 days in *Philautus leucorhinus* (Gururaja and Ramachandra, 2005).

The temperate anurans from higher elevations have delayed maturity, a larger size at maturity and slower growth rate as compared with frogs at lower elevations (Lu *et al*., 2006). The tadpoles of *Rana limnocharis* are unable to survive at very low temperature (5°C) and higher temperature (28°C or more), but they are well adapted at temperature range of 14°C to 22°C. The developing embryo took 36 days to become a froglet (Roy and Khare, 1978).

The importance of thyroid hormones in amphibian metamorphosis is well established fact (Shi, 2000; Downie *et al*., 2004). The thyroid hormones induce the complete metamorphosis of anuran tadpoles in to juvenile frogs. It has been reported that equine thyroid extracts could accelerate the metamorphosis of tadpoles into juvenile frogs. In tadpoles of *Xenopus leaves* metamorphosis, 3, 5, 3′- triiodothyronine (T₃) is more potent than 3, 5, 3′, 5′- tetraiodothyronine (T₄) (Furlow and Neff, 2006). Temperature-mediated morphological changes during metamorphic climax have been reported in the African clawed frog, *Xenopus laevis* (Walsh *et al*., 2008). The larval development in *Ascaphus* reportedly depends upon the effect of both temperature and thyroxine (T₄) (Brown, 1990). The interactions of temperature and steroid hormones on larval growth, development, and metamorphosis in a toad (*Bufo boreas*) were found to be age-dependent (Hyaes *et al*., 1993). In *Rana pipiens*, tail resorption and hind limb growth and development were induced by immersion in T₄ and accelerated under longer photoperiods and continuous light (Wright *et al*., 1988; Lehman, 1997). Red light has been found to accelerate metamorphosis in the Indian skipper frog, *Rana cyanophlyctis* in comparison to white light or other colors.
of light (Joshi and Mohinuddin, 2003). However, addition of melatonin to aquarium water prevented the red-light induced acceleration of metamorphosis. The mechanism by which red light accelerated metamorphosis is not yet known. Perception of red light through pineal gland modulates ovarian function in the skipper frog, *Rana cyanophlyctis* (Joshi and Mohinuddin, 2003).

It has been seen that the metamorphosis in African clawed frog, *Xenopus laevis* slows below $18^\circ$C and was maximum during $25.5-32^\circ$C (Downie et al., 2004). Published data suggest that amphibian metamorphosis at higher altitudes and latitudes tend to have shorter activity periods and hence shorter breeding seasons, have longer larval periods and larger size at all larval stages, are larger as adults, reach reproductive maturity at older ages, produce fewer clutches per year, produce larger clutches absolutely and smaller clutches relative to the body size and produce larger eggs (Morrison and Hero, 2003).

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 are 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. These findings can be used in planning the conservation of the frog under its natural habitats and its breeding under captive conditions.
Plate 3.1: Developmental stages (Gosner stages 1 – 9) of *Rana leptoglossa.*
Plate 3.2: Developmental stages (Gosner stages 10 – 20) of Rana leptoglossa.
Plate 3.3: Developmental stages (Gosner stages 21 - 26) of *Rana leptoglossa.*
Plate 3.4: Developmental stages (Gosner stages 31 - 42) of *Rana leptoglossa*.
Fig. 3.28: Hind limb with five toes (Gosner Stage 31-39)

Fig. 3.29: Fore limb with four fingers (Gosner Stage 40-42)

Plate 3.5: Limbs (Gosner stages 26-42) of *Rana leptoglossa*. 
Plate 3.6: Developmental stages (Gosner stages 44 - 46) of *Rana leptoglossa*. 
Plate 3.7: Life cycle of *Rana leptoglossa*.

REFERENCES:
