

CHAPTER - I

NORMAL TABLE OF DEVELOPMENT

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Introduction

Bufo himalayanus is an endemic species of toad exists solely in the high altitude of North-Eastern Himalaya. The animals are prevalent at the different areas of Greater Himalayan range. The specimen occurs in large number and breeds in stagnant pools of rain water under natural condition.

Under laboratory condition final duration of metamorphosis, on an average, is two and half a month with temperature preferably between 15^o-20^oC. While the metamorphosis time taken can be shortened under natural condition having relatively higher temperature between 22^o-24^o C.

The changing morphology of embryos, specially during organogenesis, necessitates a method of quantifying the progress of development. Tables of normal stages of development have been worked out for a number of species of amphibians; normal developmental tables of *Ichthyophis* (Sarasin, 1980); number of species of salamanders and few species of anurans have been vividly described by various authors during 1887 to 1981. So nearly a century old tradition of investigating larval development reached its peak of accuracy at recent time.

What is normal table ?

Complete tables of development are necessary for accurate comparison of developmental stages of larvae in different organisms. Identification of each stage must be accompanied by a given temperature; the stages then can be identified mostly by external features.

During cleavage, the stages are identified from the number and size of the blastomeres. During gastrulation, the shape of the blastopores is used as an identifying character. After gastrulation the neural plate provides easily recognizable features. During the process of organogenesis, the progress in the formation of the tail, limbs, gills and mouth are convenient features.

Development is, of course, continuous, and the designated stages gradually grade into one another. The duration of time between two successive stages varies by different factors naturally.

Gosner (1960) generalized and formatted the stages of larval development in anurans, which is still accepted as a standard Normal Table of development in any anuran species. This table is basically applicable to taxa that have aquatic eggs and larvae.

Therefore my present study on the normal larval developmental stages of *Bufo himalayanas* is made according to the Standard Normal Table proposed by Gosner (1960).

Review of normal table studies

Various types of investigations have been made by different investigators regarding egg development, hatching and post-hatching developments of anuran larvae since a very long period of time. Physiology of anuran hatching mechanism was described by Miganti and Azzolina as long before as 1955. Presence of anti-proteolytic factors in embryo also reported in 1948 by Wu *et al.*, in 1965 described different hatching enzymes at different stages of development in *Rana pipiens*. Anurans in which later development occurs in a capsule, hatch first from the vitelline membrane and much later from the capsule (Salthe, 1963). Different types and patterns of hatching are also described by a number of investigators from as early as 1905 in *Xenopus laevis* by Bles; in *Bufo bufo* by Kobayashi (1954). In other anurans this has been investigated (Kobayashi, 1954; Volpe *et al.*, 1961; Kenny, 1968; Petranka *et al.*, 1982). A number of embryologists have extensively investigated the amphibian external eggs for both descriptive and experimental studies (Duellman and Trueb, 1986).

The morphological types of anuran larvae were described earlier by Orton (1953). Her classification of anurans was oversimplified and not related with correct phylogeny (Wassersug, 1984; Wassersug and Duellman, 1984) it was used by many workers at that time. Mechanism of buccal pump in different species of anuran larvae was described by Kenny (1969) and Wassersug (1972, 1980). Mouth disc along with labial papillae and denticles were thoroughly investigated and reported by Lutz and Orton (1946), Wager (1965) and others. Morphology of larval ventral sucker and its

adaptations were observed by Nobel (1929); Duellman and Lynch (1969). Food taking behaviour of larval anurans and mouth adaptations were investigated by Altig and Brodie (1972); Gradwell (1973) and Wassersug (1980).

The most comprehensive treatment of amphibian development within a broad biological context is seen in the work of Salthe and Mecham (1974). It has been further appeared that a transition to a predominantly terrestrial life is accompanied by complete absorption of gills as observed by Harrison (1969) and Atkinson and Just, (1975) in *Rana*.

The transition from larva to adult is dramatic in anurans and definite changes take place during metamorphosis. The changes are most extensive in anurans in which the aquatic larvae undergo drastic transformation into a terrestrial adult or sub-adult.

From their larval forms of different stages it was evident that during development of larvae into a sub-adult a lot of morphological changes occur. The hind limb grows and matures. The fore limb develops within branchial chambers. The internal gills and associated blood vessels degenerate; lungs and pulmonary ventilation develop. The tail is resorbed. The skin thickens with glandular developments. Larval mouth parts degenerates and the adult mouth parts are formed. The eyes enlarge and eyelids develop. A comprehensive summary of the morphological changes during metamorphosis were described by Dodd and Dodd (1976) and Fox (1981,1984), Reilly et al, (1994) (Table-2).

TABLE - 2**Summary of some metamorphic changes in anurans**

System	Larva	Adult
Locomotory	Aquatic, tail fin	Terrestrial, tail-less tetrapod
Respiratory	Gills, skin, lungs; larval hemoglobins	Skin, lungs; adult hemoglobins
Circulatory	Aortic arches, aorta , anterior, posterior and common jugular veins	Carotid arch, systemic arch, cardinal veins
Nutritional	Herbivorous: long spiral gut; intestinal symbionts, small mouth, horny jaws, labial teeth	Carnivorous: short gut, proteases, large mouth with long tongue
Nervous	Lack of nictitating membrane, porphyropsin, lateral line system, Mauthner's neurons	Development of ocular muscles, nictitating membrane, rhodopsin, loss of lateral line system, degeneration of Mauthner's neurons, tympanic membrane
Excretory	Largely ammonia, some urea (ammonotelic)	Largely urea, high activity of enzymes of ornithine-urea cycle (Ureotelic)
Integumental	Thin, bi-layered epidermis with thin dermis, no mucous glands or granular glands	Stratified squamous epidermis with adult keratins, well developed dermis contains mucous glands and granular glands secreting anti-microbial peptides

Data from: Turner and Bagnara , 1976 and Reilly et. al., 1994.

Aims and objectives of the present study

Although the occurrence of several amphibians as well as anuran species from the different altitude of Himalayas had been investigated and described earlier by a host of investigators (Anandale, 1909; Bhatt, 1969; Danial, 1963; Limbaugh, and Volpe, 1957 and Smith, 1924) but a comprehensive study on the life history of *Bufo himalayanus*, specially from north-Eastern Himalayas was yet to be done.

The present investigation thus endeavours to draw a detailed account of larval development of *Bufo himalayanus* collected from Darjeeling, West Bengal from their natural habitat and breeding places, on the basis of the relationship between various body measurements to the total length according to procedure described by Martin and Little, (1966).

In the present thesis stages of developing larvae with special reference to their external feature changes during the course of metamorphosis had been described with various visual aids for easier comparative study of the normal table of this endemic, endangered, high altitude species. An attempt has been made to compare the life tables of various anuran species, particularly with that of related species of the same genus, *Bufo* (Khan, 1964)

Methodology

Different stages of developing larvae of *Bufo himalayanas* along with their eggs were collected from Sinchal Lake , Shrubbery Park, Happy Valley Tea Estate and from its adjacent areas of Darjeeling town. Specimens also collected from a few stagnant water logged places in and around the town . Sufficient amount of water was also collected from the natural habitat.

Larvae of *Bufo himalayanas* occur along with the other types of anurans ; for example, *Rachophorus* sp. and *Rana* sp. . Therefore the type under investigation were sorted out in the laboratory. The eggs of these species were laid on a gelatinous ribbon-like strips. These collected egg ribbons were kept in separate aquarium for studying the early stages of larval development. Further rearing at laboratory was made at room temperature on and around 18 degree centigrade. It was found that the larvae thrive well at a higher temperature but below 22°C. Rearing of the larvae were carried out in spacious aquariums partly filled with water collected from the collection spot.

Rearing of different stages of developing larvae were carried out in different enamel and plastic trays containing water collected from their natural habitat. Later stages of development were carried out within plastic trays to prevent extra chill of the metallic trays particularly at night. Furthermore during cold night warmth of hanging electric lamps were provided to the developing larvae.



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Though the aquarium water of the larvae was provided with same vegetation and microorganisms present in their natural habitat, an artificial food for the larvae was also offered periodically. This artificial food was made with thoroughly mixed boiled lettuce, dry fish food and dry powdered fish together.

The larvae hatched out from the fertilized eggs were allowed to develop into early larval stages. They are now transferred to plastic trays to develop further. On every second / third day certain number of individuals were fixed in 4% formalin. The pigmentation of these larvae is high and thus dark black coloration obstruct perfect visualization and observation. Therefore instead of alcohol preservation, I have preferred formalin preservation to diminish the pigmentation to some extent.

Larvae collected from the natural habitat were also allowed to develop in separate trays to compare the specific identity of the different stages of the larvae. The adults were preserved in 70-80% alcohol.

Measurements of different larval stages were taken with the help of mm/cm scale and compass uniformly. Changes in the external features in accordance with the post-hatching larval stages were made following the method described by Gosner (1960) for subsequent observations.

Camera lucida drawings were made with simple and binocular microscope from stage-20 to stage 46. Photographs were also taken from binocular optical microscope with artificial light controls. Special emphasis was given to the development of limbs ,tail and mouth disc. Regression of tail was also recorded.

During this development different stages of larvae emerged and gradually developed into the next stages. These changes are descriptively summarized in a normal table of development.

OBSERVATION

[A] FIELD OBSERVATIONS:

(i) Behaviour

(a) Adult

The specimens were found in shallow water at about two feet depth. Some preferred to remain at the surface water. Very often they were found in small waterlogged pools with stagnant water provided with aquatic vegetations. Adult male and female were found in pairs. But in a small pool of water they were found in scanty numbers. Females are comparatively larger in size with reddish prominent pigmentation in the ventral surface of the body at breeding season. The breeding season starts from April and continues till August. During breeding season their courtship begins. Fertilization is external like other anurans. Therefore courtship only referred to the fertilization process. The males comes by the side of the water bodies and starts producing mating calls by sequential inflation of their vocal sacs. Many female toads come to the these sites being attracted by the male toad's mating calls. The male toad climb on the back of the female and clinged there by their limbs. The female starts producing strings of eggs linerely arranged on a gelatinous ribbon. Huge number of eggs were laid by a female in shallow water most of which were fertilized externally by the sperms discharged by the males over the eggs ribbons.

(b) Egg

Eggs are laid as a continuous gelatinous ribbon in linear fashion. Egg ribbons are found attached with aquatic vegetations. Most of the eggs are externally fertilized. Each egg has a central ovoid or spherical opaque body surrounded by semitransparent gelatinous mass (Plate 3, Figure a).

(c) Larva

The larvae are very sluggish in movement . They are poor swimmers due to the presence of heavier head and moderate tail. They are uniformly deep black in colour . As they are not good swimmers, they often need support of the aquatic plant or weed to remain near the surface of water. In natural as well as in laboratory condition, they are found near the surface of water at night , and often in cloudy weather probably by getting attracted by natural and artificial light. In natural condition larvae often found to stay in clutch near the surface water and never actively swim unless disturbed. The larvae are found in huge number soon after the onset of the breeding season upto the end of August which culminates the breeding season. However larvae in late October or early November are not uncommon in the field. These larvae may go for hibernation in the next stage before the onset of winter .

(ii) Feeding habit**[a] Adult and sub-adult:**

In nature adult *Bufo himalayanus* (both male and female) are usually carnivorous. With their inverted and extended tip of the tongue, they procure small insects like lepidopteran moths and butterflies, orthopterans like small to medium sized grasshopper, dipteran flies ants and almost all types of terrestrial insects. They also feed on earthworms and other types of worms, snails and larvae of insects. When habituated with aquatic life in their breeding season, they also consume small fishes aquatic insect larvae and aquatic adult insects.

Post-metamorphic juveniles or sub-adults usually consume small bivalves, various forms of collembolans and lepidopteran larvae and some terrestrial forms of isopods.

Table-3

A comparative list of food items of larvae, sub-adult and adult of <i>Bufo himalayanas</i>	
Stages	Food items
1. larvae (post-hatching)	Various bacteria, diatom, protozoa, phto- and zooplankton including cladocera, rotifera, nematocera, nematodes, chironomid larvae, tubifex, different types of algae and weeds' fragments
2. Sub-adult (post-metamorphic juvenile)	Small bivalves, various collembolans and lepidopteran larvae, small soil nematodes and some terrestrial isopods etc.
3. Adult	Small insects, viz. moths and butterflies, small to medium sized grasshoppers, dipteran flies and other small to medium sized insects, earthworms, nematodes, snails and insect larvae, aquatic insects and insect larvae, small fishes etc.

[b] Larvae

The larvae are presumably column or bottom feeder in habit. In natural habitat the larvae usually consume various bacteria, diatoms, protozoans, phyto- and zooplanktons including cladocera, rotifers, nematocera etc., which occur in the sediments of the bottom of water body.

Older larvae, however, consume several groups of soil nematodes, *Tubifex* and huge amount of chironimid larvae which are usually abundant in the natural habitat of this high altitude areas. A comparative list of food items naturally consumed by the larvae, sub-adult and adult are summarized in the Table- 3 .

[B] LABORATORY OBSERVATION

(i) Eggs

Eggs were arranged linearly and equidistantly within a common gelatinous ribbon which sometimes may be several feet long (but single if separated by mechanical manipulation shows an ovoid or spherical structure with a prominent opaque central developing embryo or ripe egg. Central egg is surrounded by semi-transparent gelatinous spherical mass (Plate 3, fig a).

PLATE 3

Legends

Figure a: An egg strip with gelatinous ribbon and a single separated egg enveloped by gelatinous mass

Figure b: Anterior part of a mature female enlarged to show the characteristic shape of its parotid gland

Figure c: Ventral side of the mature female showing bulging belly and pigmentation



FIGURE:a



FIGURE: b

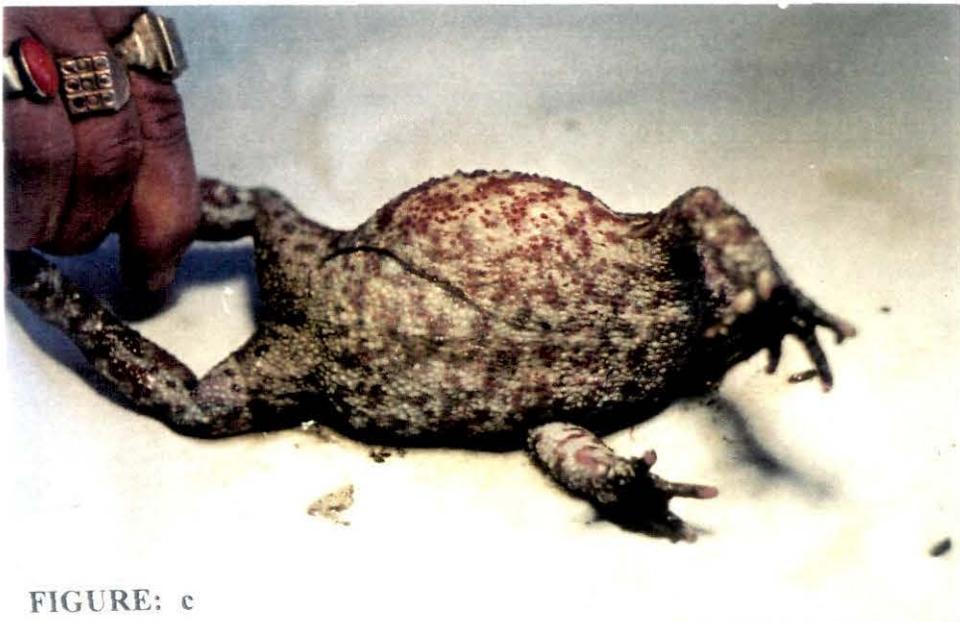


FIGURE: c

(ii) Early embryonic development

Detailed descriptions of these stages are not within our purview.

However, these stages are included in the diagram 1.

(iii) Pre-hatching embryo

The developing embryo at stage-18 are dark grey to black bodies with a remnant of yolk sac attached to the body. A ventral notch is present in between the yolk sac and the small tail bud. Gill pouch, auditory vesicle and pronephros are also developed at this stage.

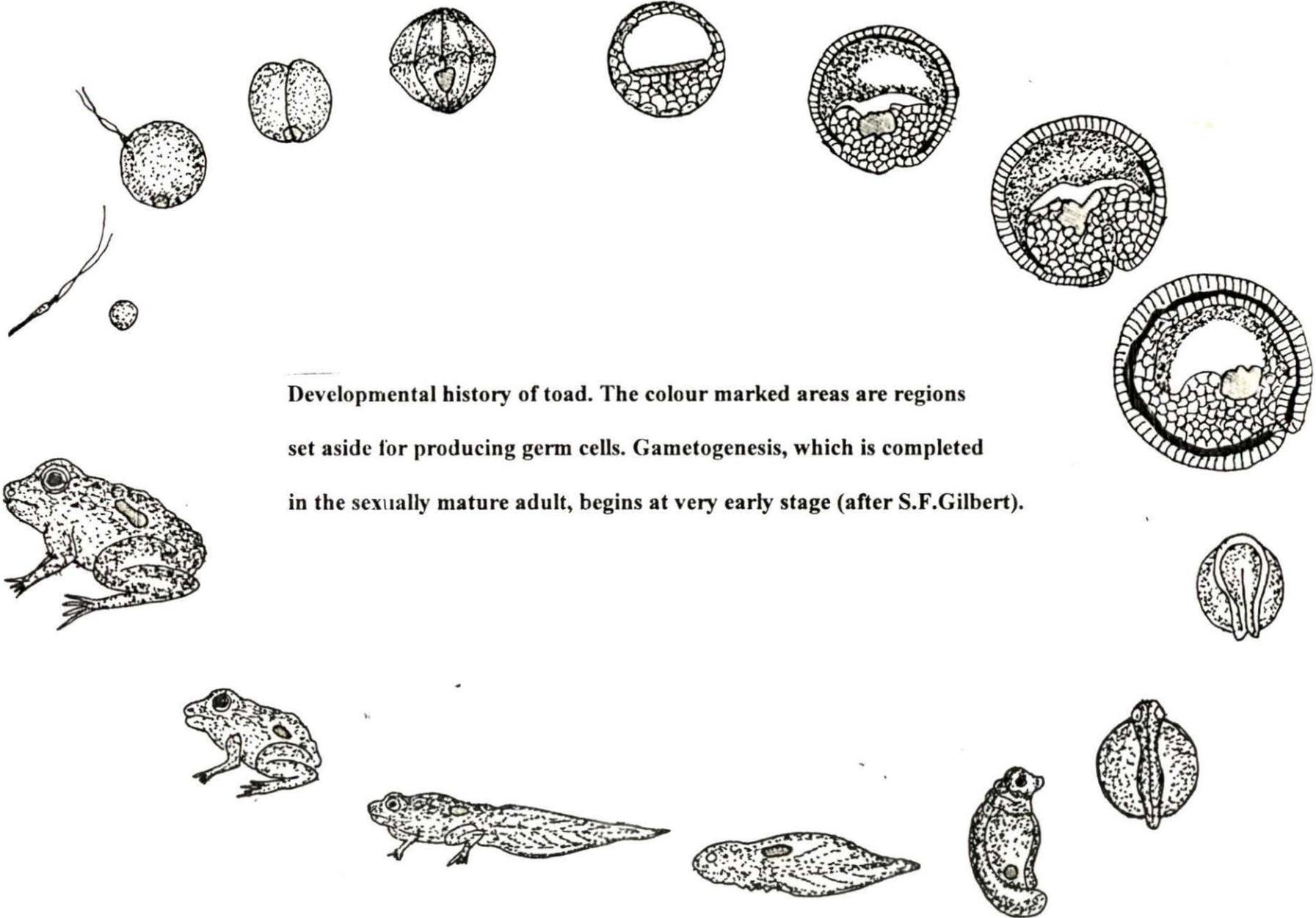
From stage -20 onwards the free living larval stages begin and the yolk sac is almost absorbed.

(iv) Post-hatching embryo

Hatching occurs at about stage-20. Due to intensive pigmentation, the larval colour is deep black. The tail fin is rather semitransparent with a round terminal end which later on became tapering and pointed at the end. Ventral sucker and three pairs of external gills are well marked from this stage.

A detailed morphological observations based on external features has been recorded in this current investigation which is followed by the measurement comparisons and ratio tables of the himalayan toad, *Bufo himalayanus*.

DIAGRAM : 1



Developmental history of toad. The colour marked areas are regions set aside for producing germ cells. Gametogenesis, which is completed in the sexually mature adult, begins at very early stage (after S.F.Gilbert).

(v) Post-hatching embryo's normal table (from stage-20)

In this current investigations description of normal table of *Bufo himalayanas* is based on the methods described and standardized by Gosner, K.L. (1960). For the present normal table here camera lucida drawings has been made of each stage of development up to post-metamorphic sub-adult stage-46. Special attention was given to the external morphology, limbs and tail development, changing pattern of mouth disc and tail regression process. Light microscopic and normal close-up photographs were also made for each individual case for a vivid and detailed study. Measurements were made in mm scale. Measurements were made on an average from at least five individual specimens of the same stage. Statistical analysis were made for each parameter, item and set as per conventional method suggested for biological specimens.

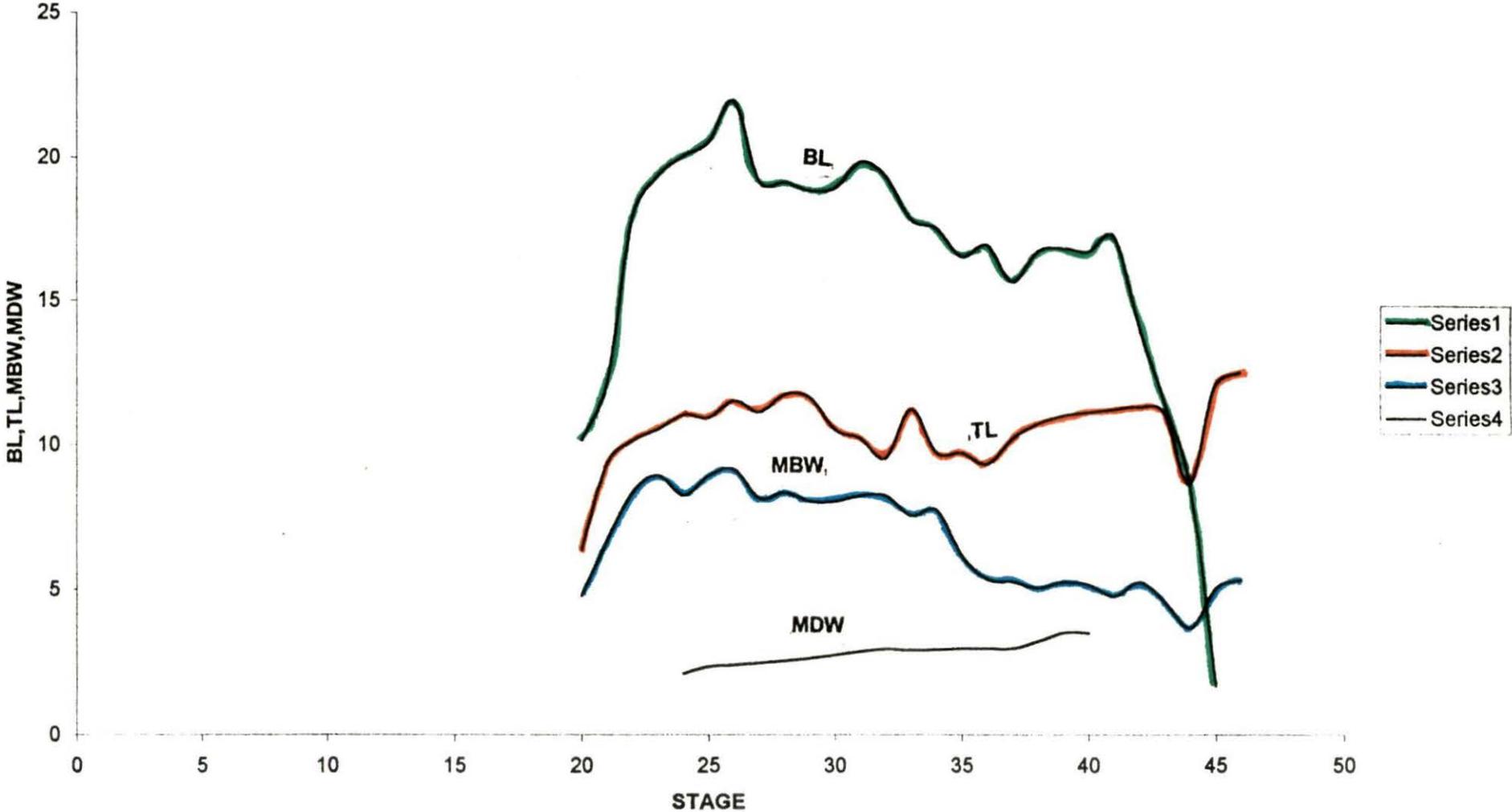
STAISTICAL INTERPRETATION

1. Body length (BL): increases steadily upto stage 29, decreases from stage 30 to 36 (not significant), remains almost stable till the end barring stage 44 when a sudden decrease occurs.
2. Tail length (TL): increases till stage 26 and then drops at stage 27, remains more or less stable till stage 32 and then decreases slowly. The decrease at stage 45 is remarkable.
3. Maximum body width (MBW): increases till stage 26 and then decreases slowly in the subsequent stages. Significant decrease starts from 33 onwards (almost linear reduction).
4. Mouth disc width (MDW): Increase starts at stage 24, then increases linearly till stage 40.

From the above statistical interpretation, I may suggest that biologically this normal table has a significant gradual and linear decrease in the tail length of the developing tadpoles. In the maximum body width and mouth disc width there is a similar significant linear reduction and linear increment respectively. This pattern of linearity may be noticeable in other related groups of anuran species. Thus I may conclude that aquatic tadpoles of anurans species shows a significant correlation in the pattern of their developmental growth.

A comparative graph (Graph-1) showing the correlation and growth pattern in *Bufo himalayanus* is given in the next page.

GRAPH 1



GRAPH 1

Periodic life table of *Bufo himalayanus*

Stage 20: Measurements:

- (i) Total length: An average of 16-17mm.
- (ii) Body length: An average of 6.4mm.
- (iii) Tail length: An average of 10.2mm.
- (iv) Max. body width: An average of 4.8mm.

Special features: Ventral sucker and three pairs of external gills present. Operculum not distinguishable. Tail membrane (fin) broad and semi-transparent with rounded tip. Dark black in colour. (Plate 4, Figure 20/ABC, Diagram 2/20).

Stage :21 Measurements:

- (i) Total length: An average of 21-22 mm.
- (ii) Body length: An average of 9.4mm.
- (iii) Tail length: An average of 12.2mm.
- (iv) Max. body width: An average of 6.7mm.

Special features: Transparent eye, elongated body, tail membrane broad, dense pigmentation, body oval in shape (Plate 4, Figure 21/ABC, Diagram 2/21).

Stage :22 Measurements:

- (i) Total length: An average of 28.2 mm.
- (ii) Body length: An average of 10.2mm.
- (iii) Tail length: An average of 18mm.
- (iv) Max. body width: An average of 8.4mm.

Special features: Tail membrane more transparent and gradually tapering towards tip, opercular fold not distinguishable, body oval in shape (Plate 4, Figure 22/ABC, Diagram 2/22).

Stage :23 Measurements:

- (i) Total length: An average of 30mm.
- (ii) Body length: An average of 10.6mm.
- (iii) Tail length: An average of 19.4mm.
- (iv) Max. body width: An average of 9mm.

Special features: Mouth opening appears, opercular fold appear, three pairs of external gills seen, tail myotomes prominent, more elongated body (Plate 5, Figure 23/ABC, Diagram 2/23).

Stage : 24 Measurements:

- (i) Total length: An average of 31.2m.
- (ii) Body length: An average of 11.1mm.
- (iii) Tail length: An average of 20.1mm.
- (iv) Max. body width: An average of 8.3mm.
- (v) Max. mouth disc width : An average of 2.12mm.

Special features: Opercular folds tend to close; mouth disc disappear, mouth is with distinct lips and denticles; tail myotomes increase in number (Plate 5, Figure 24/ABC, Diagram 2/25).

Stage : 25 Measurements:

- (i) Total length: An average of 31.6mm.
- (ii) Body length: An average of 11mm.
- (iii) Tail length: An average of 20.6mm.
- (iv) Max. body width: An average of 9mm.
- (v) Max. mouth disc width : An average of 2.36mm.

Special features: Spiracles appear, external gills disappear, mouth disc widens and get prominent jaws-like structure, eyes opaque and black (Plate 5, Figure 25/ABC, Diagram 2/26).

Stage : 26 Measurements:

- (i) Total length: An average of 33.6mm.
- (ii) Body length: An average of 11.6mm.
- (iii) Tail length: An average of 22mm.
- (iv) Max. body width: An average of 9.2mm.
- (v) Max. mouth disc width : An average of 2.42mm.

Special features: Spiracles appear, external gills disappear, mouth disc widens and get prominent jaws-like structure, eyes opaque and black (Plate 5, Figure 26/ABC, Diagram 2/27).

Stage : 27 Measurements:

- (i) Total length: An average of 30.4mm.
- (ii) Body length: An average of 11.2mm.
- (iii) Tail length: An average of 19.2mm.
- (iv) Max. body width: An average of 8.2mm.
- (v) Max. mouth disc width : An average of 2.5mm.

Special features: Hind limb bud increases in size and more or less than half half of its diameter, eyea prominent and black, body oval in shape (Plate 6, Figure 27/ABC, Diagram 2/27).

Stage : 28 Measurements:

- (i) Total length: An average of 31mm.
- (ii) Body length: An average of 11.8mm.
- (iii) Tail length: An average of 19.2mm.
- (iv) Max. body width: An average of 8.4mm.
- (v) Max. mouth disc width : An average of 2.56mm.

Special features: Hind limb bud elongates, slightly greater than its diameter, mouth widens, jaws with denticles, body oval in shape (Plate 6, Figure 28/ABC, Diagram 2/28).

Stage :29 Measurements:

- (i) Total length: An average of 30.6mm.
- (ii) Body length: An average of 11.7mm.
- (iii) Tail length: An average of 18.9mm.
- (iv) Max. body width: An average of 8.1mm.
- (v) Max. mouth disc width : An average of 2.66mm.

Special features: Hind limb bud elongates more and its length is greater than its diameter by 1.5 times; other features are as in stage-28. (Plate 6, Figure 29/ABC, Diagram 3/29).

Stage : 30 Measurements:

- (i) Total length: An average of 29.6mm.
- (ii) Body length: An average of 10.6mm.
- (iii) Tail length: An average of 19mm.
- (iv) Max. body width: An average of 8.1mm.
- (v) Max. mouth disc width : An average of 2.76mm.

Special features: Hind limb bud elongates, slightly greater than its diameter, mouth widens, jaws with denticles, body oval in shape (Plate 6, Figure 30/ABC, Diagram 3/30).

Stage : 31 Measurements:

- (i) Total length: An average of 30.2mm.
- (ii) Body length: An average of 10.3mm.
- (iii) Tail length: An average of 19.9mm.
- (iv) Max. body width: An average of 8.3mm.
- (v) Max. mouth disc width : An average of 2.88mm.

Special features: Hind limb tip flattened and conical, paddle shaped tip of hind limb without any digit, mouth with stout chitinous pair of jaws, tail with distinctly visible myotomes (Plate 7, Figure 31/ABC, Diagram 3/31).

Stage : 32 Measurements:

- (i) Total length: An average of 29mm.
- (ii) Body length: An average of 9.6mm.
- (iii) Tail length: An average of 19.4mm.
- (iv) Max. body width: An average of 8.3mm.
- (v) Max. mouth disc width : An average of 2.98mm.

Special features: Hind limb tip or foot starts digitalization, 4th and 5th digit are visible now, tail is almost twice the body length, rigid chitinous pair of jaws guards the mouth aperture, operculum is now distinct (Plate 7, Figure 32/ABC, Diagram 3/32).

Stage : 33 Measurements:

- (i) Total length: An average of 27.6mm.
- (ii) Body length: An average of 11.3mm.
- (iii) Tail length: An average of 17.9mm.
- (iv) Max. body width: An average of 7.6mm.
- (v) Max. mouth disc width : An average of 2.94mm.

Special features: More digits are distinguishable at the tip of the hind bud and this is more stout and well developed, operculum distinct, semitransparent and broad tail fin (Plate 7, Figure 33/ABC, Diagram 3/33).

Stage : 34 Measurements:

- (i) Total length: An average of 27.4mm.
- (ii) Body length: An average of 9.8mm.
- (iii) Tail length: An average of 17.6mm.
- (iv) Max. body width: An average of 7.8mm.
- (v) Max. mouth disc width : An average of 2.96mm.

Special features: Digits or toes are more distinct, 1st and 2nd toes not separated yet, tail fin or membrane not so broad, hind limb is more developed with more joints (Plate 8, Figure 34/ABC, Diagram 3/34).

Stage : 35 Measurements:

- (i) Total length: An average of 26.4mm.
- (ii) Body length: An average of 9.8mm.
- (iii) Tail length: An average of 16.6mm.
- (iv) Max. body width: An average of 6.2mm.
- (v) Max. mouth disc width : An average of 3mm.

Special features: Toes of the hind limb now distinguishable fully, all toes separated from each other, hind limb develops bends behind the foot, tail fin is thin, length of the tail is about twice the body length (Plate 8, Figure 35/ABC, Diagram 3/35).

Stage : 36 Measurements:

- (i) Total length: An average of 27.2mm.
- (ii) Body length: An average of 9.4mm.
- (iii) Tail length: An average of 17mm.
- (iv) Max. body width: An average of 5.4mm.
- (v) Max. mouth disc width : An average of 3mm.

Special features: All toes are completely separated except 1st and 2nd which are slightly joined at their base, mouth is guarded by a pair of thick lips with cuticular thickening, body slightly elongated (Plate 8, Figure 36/ABC, Diagram 3/36).

Stage : 37 Measurements:

- (i) Total length: An average of 26mm.
- (ii) Body length: An average of 10.3mm.
- (iii) Tail length: An average of 15.7mm.
- (iv) Max. body width: An average of 5.3mm.
- (v) Max. mouth disc width : An average of 3mm.

Special features: All 5 toes are free and completely separated, hind limb develops more joints and bends, mouth aperture broadens, body tail ratio is about 1:2 in length (Plate 9, Figure 37/ABC, Diagram 3/37).

Stage : 38 Measurements:

- (i) Total length: An average of 27.6mm.
- (ii) Body length: An average of 10.8mm.
- (iii) Tail length: An average of 16.8mm.
- (iv) Max. body width: An average of 5.1mm.
- (v) Max. mouth disc width : An average of 3.24mm.

Special features: Hind limbs develop inner metatarsal tubercle, mouth widens more, body length is almost as in previous stage, tail membrane thin (Plate 9, Figure 38/ABC, Diagram 4/38).

Stage : 39 Measurements:

- (i) Total length: An average of 28mm.
- (ii) Body length: An average of 11.1mm.
- (iii) Tail length: An average of 16.9mm.
- (iv) Max. body width: An average of 5.3mm.
- (v) Max. mouth disc width : An average of 3.54mm.

Special features: A whitish patch develop on ventral surface of toes and these pigment free patches will develop subarticular tubercles, body and tail length remains at 1:2 ratio (Plate 9, Figure 39/ABC, Diagram 4/39).

Stage : 40 Measurements:

- (i) Total length: An average of 28mm.
- (ii) Body length: An average of 11.2mm.
- (iii) Tail length: An average of 16.8mm.
- (iv) Max. body width: An average of 5.2mm.
- (v) Max. mouth disc width : An average of 3.52mm.

Special features: Formation of hind limb almost completed, subarticular tubercles formed on toes, mouth disc now widens maximum, cloacal tail piece present, fore limbs develop within opercular folds (Plate 10, Figure 40/ABC, Diagram 4/40).

Stage : 41 Measurements:

- (i) Total length: An average of 28.6mm.
- (ii) Body length: An average of 11.3mm.
- (iii) Tail length: An average of 17.3mm.
- (iv) Max. body width: An average of 4.8mm.
- (v) Max. mouth disc width : An average of 3.24mm.

Special features: Skin over fore limb thin and transparent, fore limb almost developed within the skin, larval mouth parts starts degeneration, tail starts regression (Plate 10, Figure 41/ABC, Diagram 4/41).

Stage : 42 Measurements:

- (i) Total length: An average of 25.6mm.
- (ii) Body length: An average of 11.4mm.
- (iii) Tail length: An average of 14.2mm.
- (iv) Max. body width: An average of 5.3mm.

Special features: Fore limb protrude out of the skin , fore limb almost developed fully, angle of mouth changed, mouth opening shifted upward anterior to nostril, horny beak like larval mouth disappear (Plate 10, Figure 42/ABC, Diagram 4/42).

Stage : 43 Measurements:

- (i) Total length: An average of 22.8mm.
- (ii) Body length: An average of 11.2mm.
- (iii) Tail length: An average of 11.6mm.
- (iv) Max. body width: An average of 4.6mm.

Special features: Mouth widens laterally, angle of mouth rests between nostril and midpoint of eye, bony jaws and muscular tongue formed, tail regression is more (Plate11, Figure 43/ABC, Diagram 4/43).

. Stage : 44 Measurements:

- (i) Total length: An average of 17.4mm.
- (ii) Body length: An average of 8.7mm.
- (iii) Tail length: An average of 8.7mm.
- (iv) Max. body width: An average of 3.7mm.

Special features: Mouth widens further, angle of mouth rests at the end point of eye, body length and tail almost equal (1:1), tail further regressed to a small one, bulging eye developed (Plate 11, Figure 44/ABC,Diagram 4/44).

Stage : 45 Measurements:

- (i) Total length: An average of 13.8mm.
- (ii) Body length: An average of 12.1mm.
- (iii) Tail length: An average of 1.7mm.
- (iv) Max. body width: An average of 5.1mm.

Special features: Mouth widens to its maximum, angle of mouth crosses the eye, regression of tail is almost complete and reduced to a stub, fully developed fore and hind limbs with digits (Plate 11, Figure 45/ABC, Diagram 4/45).

Stage : 46 Measurements:

- (i) Total length: An average of 12.4mm.
- (ii) Body length: An average of 12.6mm.
- (ii) Max. body width: An average of 5.4mm.

Special features: Tail completely resorbed, process of metamorphosis is completed, slightly elongated body with all internal and external organs, hind limbs more stout and more muscular than the fore limbs, grayish in color, a small toad which resembles the adult except the size, starts its terrestrial life (Plate 12, Figure A B C D, Diagram 4/46).

To summarize, in the himalayan toad (*Bufo himalayanus*) hatching starts at about stage-20, in laboratory condition. At this stage the larvae attains a length of 16-17 mm. Ventral sucker, olfactory pits and eyes are visible. Tail membrane is transparent. Three pairs of gills gradually get within the opercular folds. From stage 23 mouth disc starts developing and from this stage larvae begin its free swimming and feeding life. Development of mouth parts is completed by stage 25. Horney beak-like larval jaws are developed at and around stage 25 (Plate 5, Figure 25/ABC).

At the stage 26, the hind limb bud appears. The average measurements during these periods are described in the Table 3. Limb buds gets prominent shape at the stage 29. Up to stage 40 hind limb develops into its climax and size of the larvae gradually increases. Hind limb formation is completed at the end of stage 38. The larvae at stage 40 show deep black pigmentation both on dorsal and ventral surfaces. The myotomes of the tail with black pigmentation along the dorsal and ventral side is also observed (Plate 10, Figure 40/ABC,). Tail membrane becomes brown in color. Cornea tends to dilated and becomes larger than the eye ball itself. The mouth is sub-terminal with one upper and two lower rows of larval denticles. Body proportion of individual larva from stage 26-40 remain constant to some extent in *Bufo himalayanus* (Table 4 & 5: Plate 5-10). These findings are very similar as *Bufo valiceps* (Limbaugh and Volpe, 1957).

Various body dimensions and body proportions of different stages of larvae are summarized in Table 4. Some unexpected results in the data may occur due to ill-fed or undernourished forms or due to specimens having stunted growth by some intrinsic and extrinsic factors in the laboratory conditions.

Total duration of the metamorphosis on an average is about two and a half months in the species described. However, this time of total metamorphosis can be shortened under higher temperature and natural conditions.

Fore limb protrusion out of the skin takes place in stage-42. Adult mouth complexity replaced the larval mouth parts at stage-44. Tail resorbed completely in stage-46. This stage is called sub-adult where metamorphosis is fully completed.

Drastic morphological changes occur mainly from stage 41 to stage 46. (Plate 10-12, Diagram 4).In other words, it can be said that metamorphosis is initiated at stage at stage 41 and completed by stage 46. In these four stages drastic changes took place and the animal dramatically changes its habitat, from aquatic to terrestrial life.

Periodic life table of *Bufo himalayanas*

Periodic life table of *Bufo himalayanas*

Table No.4

Stage	Total length	Body length	Tail length	Max. body width	Mouth disc width	Special features
	Units Av.	Units Av.	Units Av.	Units Av.	Units Av.	
20	16 17 16 (16.6) 18 16	6 7 6 (6.4) 7.5 5.5	10 10 10 (10.2) 10.5 10.5	5 4 5 (4.8) 5 5	Ventral sucker and three pairs of external gills present. Operculum not distinguishable. Tail membrane(fin) broad and semitransparent with rounded lip (Plate 4, Figure 20/ABC, Diagram 2/20)
21	23 20 22 (21.8) 23 21	10 9 9.5 (9.4) 10 8.5	13 11 11.5 (12.5) 13 12.5	7 6 7 (6.7) 7 6.5	Transparent eye, elongated body, tail membrane broad, dense pigmentation and body oval in shape (Plate 4, Figure 21/ABC, Diagram 2/21)
22	29 27 29 (28.2) 28 28	11 10 9 (10.2) 10 10	18 17 19 (18.0) 18 18	8.5 8 8 (8.4) 8.5 8.5	-----	Tail membrane more transparent and gradually tapering towards tip, opercular fold not distinguishable, body oval in shape (Plate 4, Figure 22/ABC, Diagram 2/22)

Periodic life table of *Bufo himalayanas*

Table No.4

Stage	Total length	Body length		Tail length		Max. body width		Mouth disc width		Special features	
	Units	Av.	Units	Av.	Units	Av.	Units	Av.	Units		Av.
23	30		11		19		9			Mouth opening appears, opercular fold appear, three pairs of external gills seen, tail myotomes prominent, more elongated body (Plate 5, Figure 23/ABC, Diagram 2/23).	
	30		11		19		9				
	31	(30.0)	10	(10.6)	20	(19.4)	9.5	(9.0)	-----		
	30		10		20		9				
	29		10		19		8.5				
24	30		11		19		8		2	Opercular folds tend to close; mouth disc disappears, mouth with distinct lips and denticles; tail myotomes increase in number (Plate 5, Figure 24/ABC, Diagram 2/24).	
	32		11.5		20.5		8		2		
	32	(31.2)	10	(11.1)	21	(20.1)	7	(8.3)	2.4		(2.12)
	30		10.5		19.5		8.5		2.2		
	32		10.5		20.5		8		2		
25	31		10		21		9		2.5	Spiracles appear, external gills disappear, mouth disc widens and get prominent jaws-like structure, eyes opaque and black (Plate 5, Figure 25/ABC, DiaGRAM 2/25)..	
	32		11.5		20.5		9		2.3		
	32	(31.6)	11	(11.0)	22	(20.6)	32	(9.0)	2.4		(2.36)
	32		11.5		20.5		9.5		2.3		
	31		11		20		8.5		2.3		

contd.

Periodic life table of *Bufo himalayanas*

Table No.4

Stage	Total length	Body length	Tail length	Max. body width	Mouth disc width	Special features
	Units Av.	Units Av.	Units Av.	Units Av.	Units Av.	
26	33	11	22	9.5	2.4	Emergence of hind limb buds, mouth widens with jaws, spiracles shifting to the ventral side of the body (Plate 5, Figure26/ABC, Diagram 2/26)..
	34	12	22	9	2.5	
	33 (33.6)	12 (11.6)	23 (22.0)	2.5 (9.2)	2.4 (2)	
	33	12	21	9	2.4	
	34	11	23	9	2.4	
27	31	11	20	8	2.5	Hind limb bud increases in size and more or less than half of its diameter, eyes prominent and black, body oval in shape (Plate 6, Figure 27/ABC, Diagram 2/27).
	30	11	19	8.5	2.6	
	29 (30.4)	15 (11.2)	18.5 (19.2)	8.5 (8.2)	2.4 (2.5)	
	31	11.5	19.5	8	2.4	
	30	11	19	8	2.5	
28	31	12	19	8.5	2.5	Hind limb bud elongates, slightly greater than its diameter, mouth widens, jaws with denticles, body oval in shape (Plate 6, Figure 28/ABC, Diagram 2/28).
	30	11.5	18.5	8.5	2.6	
	30 (31.0)	11.8 (11.8)	18 (19.2)	8 (8.4)	2.5 (2.56)	
	31	11.5	19.5	8.5	2.6	
	31	12	19	8.5	2.5	

contd.

Periodic life table of *Bufo himalayanas*

Table No.4

Stage	Total length		Body length		Tail length		Max. body width		Mouth disc width		Special features
	Units	Av.	Units	Av.	Units	Av.	Units	Av.	Units	Av.	
29	31		11.5		19.5		8		2.6		Hind limb bud elongates more and its length is greater than its diameter by 1.5 times; other features are as in stage-28. (Plate 6, Figure 29/ABC, Diagram 3/29).
	30		11.5		18.5		8		2.7		
	30	(30.6)	10.5	(11.7)	18.5	(18.9)	8.5	(8.1)	2.6	(2.66)	
	31		12		19		8		2.6		
	31		12		19		8		2.7		
30	29		11		18		8		2.7		Hind limb bud elongates, slightly greater than its diameter, mouth widens, jaws with denticles, body oval in shape (Plate 6, Figure 30/ ABC, Diagram 3/30).
	30		10.5		19.5		8.5		2.8		
	30	(29.6)	11	(10.6)	19	(19.0)	8	(8.1)	2.7	(2.76)	
	29		10		19		8		2.7		
	30		10.5		19.5		8		2.8		
31	30		10.5		19.5		8.5		2.8		Hind limb tip flattened and conical, paddle shaped tip of hind limb without any digit, mouth with stout chitinous pair of jaws, tail with distinctly visible myotomes (Plate 7, Figure 31/ABC, Diagram 3/31).
	29		10		19		8		2.9		
	31	(30.2)	10.5	(10.3)	21.5	(19.9)	8.5	(8.3)	2.8	(2.88)	
	31		10.5		20.5		8.5		3		
	29		10		19		8		2.9		

contd.

Periodic life table of *Bufo himalayanas*

Table No.4

Stage	Total length		Body length		Tail length		Max. body width		Mouth disc width		Special features
	Units	Av.	Units	Av.	Units	Av.	Units	Av.	Units	Av.	
32	29		10		19		8		3		Hind limb tip or foot starts digitalization, 4 th and 5 th digit are visible now, tail is almost twice the body length, rigid chitinous pair of jaws guards the mouth aperture, operculum is now distinct (Plate 7, Figure 32/ABC, Diagram 3/32).
	30		10		20		8		3		
	29	(29)	9.5	(9.6)	19.5	(19.4)	8.5	(8.3)	2.9	(2.98)	
	28		9		19		8.5		3		
	29		9.5		19.5		8.5		3		
33	28		9.5		18.5		8		3		More digits are distinguishable at the tip of the hind bud and this is more stout and well developed, operculum distinct, semitransparent and broad tail fin (Plate 7, Figure 33/ABC, Diagram 3/33).
	27		9.5		17.5		7.5		3		
	28	(27.6)	9	(11.3)	18	(17.9)	7.5	(7.6)	2.9	(2.94)	
	28		9.5		18.5		7.5		3		
	27		9		17		7.5		2.8		
34	28		10		18		7.5		3		Digits or toes are more distinct, 1 st and 2 nd toes not separated yet, tail fin or membrane not so broad, hind limb is more developed with more joints (Plate 7, Figure 33/ABC, Diagram 3/34).
	27		10		17		8		2.9		
	27	(27.4)	9.5	(9.8)	17.5	(17.6)	8	(7.8)	2.9	(2.96)	
	28		10		18		8		3		
	27		9.5		17.5		7.5		3		

contd.

Periodic life table of *Bufo himalayanas*

Table No.4

Stage	Total length	Body length		Tail length		Max. body width		Mouth disc width		Special features	
	Units	Av.	Units	Av.	Units	Av.	Units	Av.	Units		Av.
35	27		10		17		6.5		3	Toes of the hind limb now distinguishable fully, all toes separated from each other, hind limb develops bends behind the foot, tail fin is thin, length of the tail is about twice the body length (Plate 8, Figure 35/ABC, Diagram 3/35).	
	26		9.5		16.5		6		3.2		
	27	(26.4)	9	(9.8)	17	(16.6)	6	(6.2)	3		(3.0)
	26		9.5		16.5		6.5		3		
	26		10		16		6		2.9		
36	27		10		17		5.5		3.0	All toes are completely separated except 1 st and 2 nd which are slightly joined at their base, mouth is guarded by a pair of thick lips with cuticular thickening, body slightly elongated (Plate 8, Figure 36/ABC, Diagram 3/36).	
	28		9.5		18.5		6		2.9		
	26	(27.2)	9.6	(9.4)	17	(17.0)	4	(5.4)	3.2		(3.0)
	28		9		16.5		5.5		2.9		
	29		9		16		5		3		
37	26		10.5		15.5		5.5		3.2	All 5 toes are free and completely separated, hind limb develops more joints and bends, mouth aperture broadens, body tail ratio is about 1:2 in length (Plate 9, Figure 37/ ABC, Diagram 3/37).	
	27		10		17		5.5		3		
	26	(26.0)	10.5	(10.3)	15.5	(15.7)	5	(5.3)	3.2		(3.0)
	25		10		15		5		3		
	26		10.5		15.5		5.5		3		

contd.

Periodic life table of *Bufo himalayanas*

Table No.4

Stage	Total length		Body length		Tail length		Max. body width		Mouth disc width		Special features
	Units	Av.	Units	Av.	Units	Av.	Units	Av.	Units	Av.	
38	27		10.5		16.5		5		3		Hind limbs develop inner metatarsal tubercle, mouth widens more, body length is almost as in previous stage, tail membrane thin (Plate 9, Figure 38/ABC, Diagram 4/38).
	28		11		17		5		3.6		
	27	(27.6)	10	(10.8)	16	(16.8)	5.5	(5.1)	3.3	(3.24)	
	28		11.5		16.5		5		3		
	28		11		17		5		3.2		
39	27		11		16		5		3.9		A whitish patch develop on ventral surface of toes and these pigment free patches will develop sub-articular tubercles, body and tail length remains at 1:2 ratio (Plate 9, Figure 39/ABC, Diagram 4/39).
	27		11.5		17.5		5.5		3.6		
	28	(28.0)	11.5	(11.1)	16.5	(16.9)	4	(5.3)	3.4	(3.54)	
	28		11.5		16.5		5.5		3.6		
	28		10		18		5.5		3.2		
40	27		11		16		5		3.4		Formation of hind limb almost completed, sub-articular tubercles formed on toes, mouth disc now widens maximum, cloacal tail piece present, fore limbs develop within opercular folds (Plate 10, Figure 40/ABC, Diagram 4/40).
	28		11.5		16.5		5		3.6		
	29	(28.0)	12	(11.2)	17	(16.8)	4	(5.2)	3.6	(3.52)	
	28		10.5		17.5		5.5		3.4		
	29		11		18		5.5		3.6		

contd.

Periodic life table of *Bufo himalayanus*

Table No.4

Stage	Total length	Body length		Tail length		Max. body width		Mouth disc width	Special features
	Units Av.	Units Av.	Units Av.	Units Av.	Units Av.	Units Av.			
41	29	11	18	5			-----	Skin over fore limb thin and transparent, fore limb almost developed within the skin, larval mouth parts starts degenerating, tail starts regression (Plate 10, Figure 41/ABC, Diagram 4/41).	
	30	11.5	18.5	5					
	27 (28.6)	11 (11.3)	18 (17.3)	4.5 (4.8)					
	29	12	17	4.5					
	27	11	16	5					
42	25	12	13	5.5			-----	Fore limb protrude out of the skin , fore limb almost developed fully, angle of mouth changed, mouth opening shifted upward anterior to nostril, horny beak like larval mouth disappear (Plate 10, Figure 42/ABC, Diagram 4/42).	
	26	11	15	5					
	26 (25.6)	11.5 (11.4)	14.5 (14.2)	5.5 (5.3)					
	25	11	14	5					
	26	11.5	14.5	5.5					
43	23	11.5	11.5	4.5			-----	Mouth widens laterally, angle of mouth rests between nostril and midpoint of eye, bony jaws and muscular tongue formed, tail regression is more (Plate 11, Figure 43/ABC, Diagram 4/43).	
	24	12	12	5					
	22 (22.8)	12 (11.2)	11 (11.6)	4.5 (4.6)					
	23	11	12	4.5					
	22	10	11.5	4.5					

contd.

Periodic life table of *Bufo himalayanus*

Table No.4

Stage	Total length		Body length		Tail length		Max. body width		Mouth disc width		Special features
	Units	Av.	Units	Av.	Units	Av.	Units	Av.	Units	Av.	
44	18		9		9		4				Mouth widens further, angle of mouth rests at the end point of eye, body length and tail almost equal (1:1), tail further regressed to a small one, bulging eye developed (Plate 11, Figure 44/ABC, Diagram 4/44).
	17		9		8		3.5				
	17	(17.4)	8.5	(8.7)	8.5	(8.7)	3.5	(3.7)	-----		
	18		9		9		4				
	17		8		9		3.5				
45	15		13		2		5				Mouth widens to its maximum, angle of mouth crosses the eye, regression of tail is almost complete and reduced to a stub, fully developed fore and hind limbs with digits (Plate 11, Figure 45/ABC, Diagram 4/45).
	14		12		2		5.5				
	12	(13.8)	11.5	(12.1)	1.5	(1.7)	4	(5.1)	-----		
	14		12		2		5				
	13		12		1		5				
46	13		13				5.5				Tail completely resorbed, process of metamorphosis is completed, slightly elongated body with all internal and external organs, hind limbs more stout and more muscular than the fore limbs, grayish in color, a small toad which resembles the adult except the size, starts its terrestrial life (Plate 12, Figure A B C D , Diagram 4/46).
	12		12				5.5				
	12	(12.4)	13	(12.6)	----		5	(5.4)	----		
	12		13				5.5				
	13		12				5.5				

contd.

Table No.5
Periodic life table of *Bufo himalayanas*

Stage	Nos	Total length	Body length	Tail length	Body width	Mouth disc width	Body length/ Total length	Body width/ Body length	Mouth disc/ Body width
20	5	16.6 (16-18)	6.4 (5.5-7.5)	10.2 (10-10.5)	4.8 (4-5)	----	0.38	0.75	----
21	5	21.8	9.4 (20-23)	12.2 (8.5-10)	6.7 (11-13)	----	0.43	0.71	---
22	5	28.2 (27-29)	10.2 (10-11)	18.0 (17-19)	8.4 (8-8.5)	---	0.36	0.82	---
23	5	30.5 (29-31)	10.6 (10-11)	19.4 (19-20)	9.0 (8.5-9.5)	---	0.35	0.84	----
24	5	31.2 (30-32)	11.1 (10.5-11.5)	20.1 (19-21)	8.3 (8-9)	2.12 (2-2.4)	0.35	0.74	0.25
25	5	31.6 (31-32)	11.0 (10-11.5)	20.6 (20-21)	9.0 (8.5-9.5)	2.36 (2.3-2.4)	0.34	0.81	0.26

Table No.5

Periodic life table of *Bufo himalayanas*

Stage Nos	Total length	Body length	Tail length	Body width	Mouth disc width	Body length/ Total length	Body width/ Body length	Mouth disc/ Body width	
26	5	33.6 (33-34)	11.6 (11-12)	22 (21-23)	9.2 (9-9.5)	2.42 (2.4-2.5)	34	0.79	0.26
27	5	30.4 (30-31)	11.2 (11-11.5)	19.2 (18.5-20)	8.2 (8-8.5)	2.5 (2.4-2.6)	0.36	0.70	0.30
28	5	31 (26-32)	11.8 (11.5-12)	19.2 (18.5-20)	8.4 (8-8.4)	2.56 (2.5-2.6)	0.38	0.71	0.30
29	5	30.6 (30-31)	11.7 (11.5-12)	18.9 (18.5-19.5)	8.1 (8-8.5)	2.66 (2.6-2.7)	0.38	0.69	0.32
30	5	29.6 (29-30)	10.6 (10-11)	19 (18-19.5)	8 (8-8.5)	2.76 (2.7-2.8)	0.35	0.76	0.34

contd.

Table No.5

Periodic life table of *Bufo himalayanas*

Stage Nos	Total length	Body length	Tail length	Body width	Mouth disc width	Body length/ Total length	Body width/ Body length	Mouth disc/ Body width	
31	5	30.2 (29-31)	10.3 (10-10.5)	19.9 (18-19.5)	8.3 (8-8.5)	2.88 (2.8-3)	0.34	0.8	0.34
32	5	29 (28-29)	9.6 (9-10)	19.4 (19-20)	8.3 (8-8.5)	2.98 (2.9-3)	0.33	0.86	0.35
33	5	27.6 (27-28)	11.3 (9-10)	17.9 (17-18.5)	7.6 (7.5-8)	2.94 (2.8-3)	0.4	0.67	0.38
34	5	27.4 (27-28)	9.8 (7.5-10)	17.6 (17-18)	7.8 (7.5-8)	2.96 (2.9-3)	0.35	0.79	0.37

contd.

Table No.5
Periodic life table of *Bufo himalayanas*

Stage	Nos	Total length	Body length	Tail length	Body width	Mouth disc width	Body length/ Total length	Body width/ Body length	Mouth disc/ Body width
35	5	26.4 (26-27)	9.8 (9.5-10)	16.6 (16-17)	6.2 (6-6.5)	3 (2.9-3.2)	0.37	0.63	0.48
36	5	27.2 (26-28)	9.4 (9-10)	17 (16.5-18.5)	5.4 (5-6)	3 (2.9-3.2)	0.34	0.57	0.55
37	5	26 (25-26)	10.3 (10-10.5)	15.7 (15-17)	5.3 (5-5.5)	3 (3-3.2)	0.39	0.51	0.56
38	05	27.6 (27-28)	10.8 (10-11)	16.8 (16.5-17)	5.1 (5-5.5)	3.24 (3-3.6)	0.39	0.47	0.63

contd.

Table No.5

Periodic life table of *Bufo himalayanas*

Stage Nos	Total length	Body length	Tail length	Body width	Mouth disc width	Body length/ Total length	Body width/ Body length	Mouth disc/ Body width	
39	5	28 (27-29)	11.1 (10-11.5)	16.9 (16-18)	5.3 (5-5.5)	3.54 (3.2-3.9)	0.39	0.47	0.66
40	5	28 (27-29)	11.2 (10.5-12)	16.8 (16-18)	5.2 (5.5-5)	3.52 (3.4-3.9)	0.4	0.46	0.67
41	5	28.6 (27-30)	11.3 (11-12)	17.3 (16-18.5)	4.8 (4.5-5)	---	0.39	0.42	---
42	5	25.6 (25-26)	11.4 11-12	14.2 (13-15)	5.3 (5-5.5)	---	0.44	0.46	---
43	5	22.8 (22-24)	11.2 (10.5-12)	11.6 (11-12)	4.6 (4.5-5)	---	0.49	0.41	---
44	5	17.4 (17-18)	8.7 (8.5-9)	8.7 (8.5-9)	3.7 (3.5-4)	---	0.50	0.42	---
45	5	13.8 (13-15)	12.1 (11.5-13)	1.7 (1-2)	5.1 (5-5.5)	---	0.87	0.42	---
46	5	12.4 (12-13)	12.6 (12-13)	---	5.4 (5-5.5)	---	1.01	0.42	---



STAGE 20



a



STAGE 21



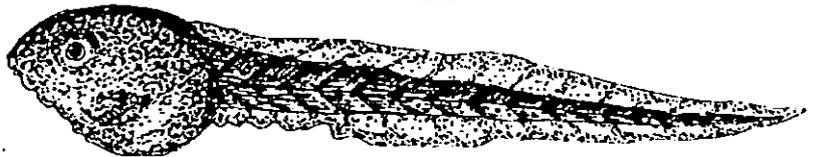
b



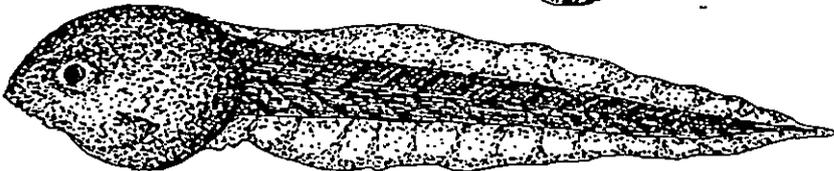
STAGE 22



d



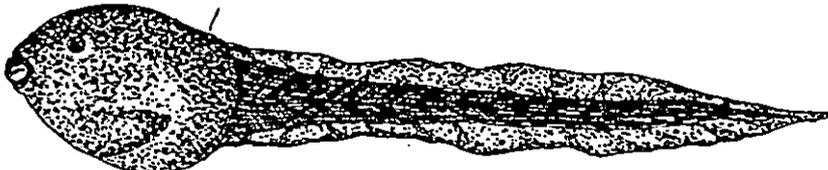
STAGE 23



STAGE 24



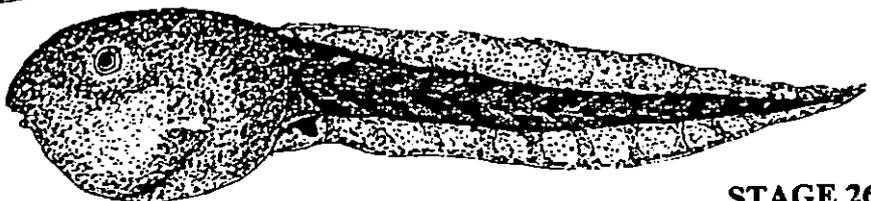
e



STAGE 25



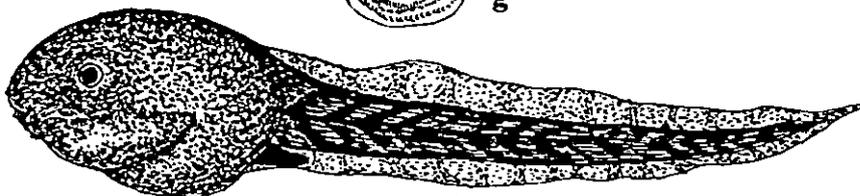
f



STAGE 26



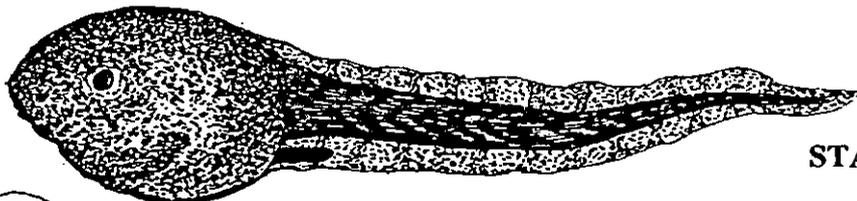
g



STAGE 27



h



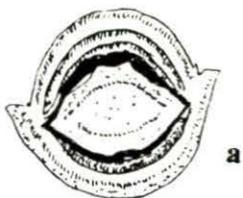
STAGE 28



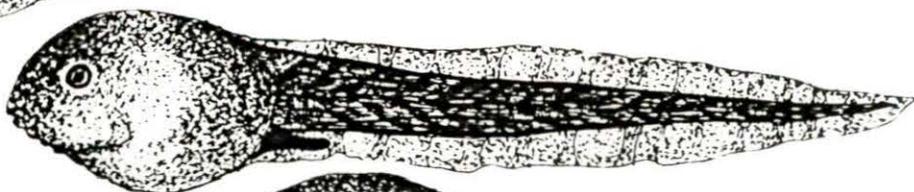
i



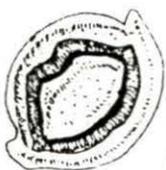
STAGE 29



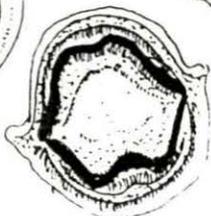
a



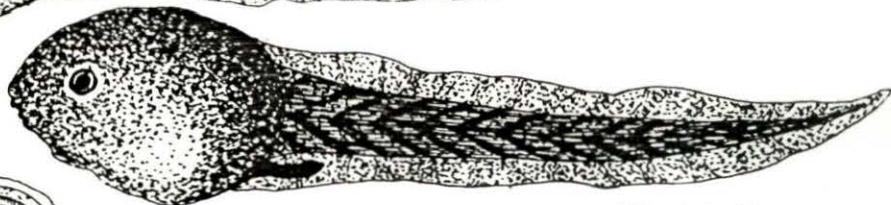
STAGE 30



b



c



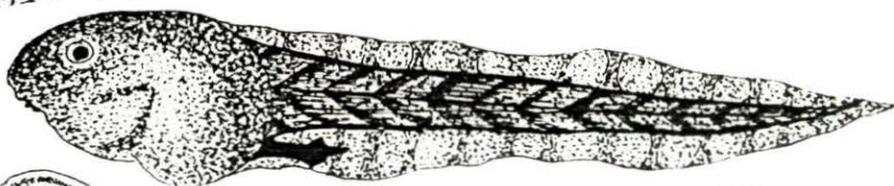
STAGE 31



STAGE 32



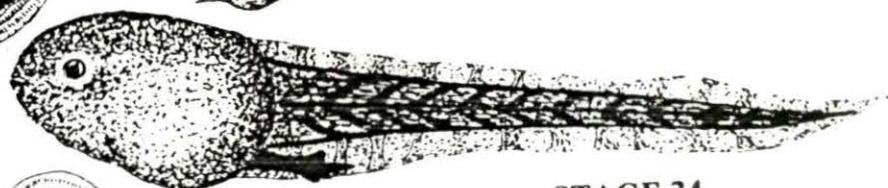
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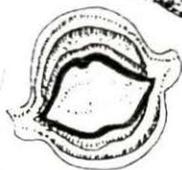
STAGE 33



e



STAGE 34



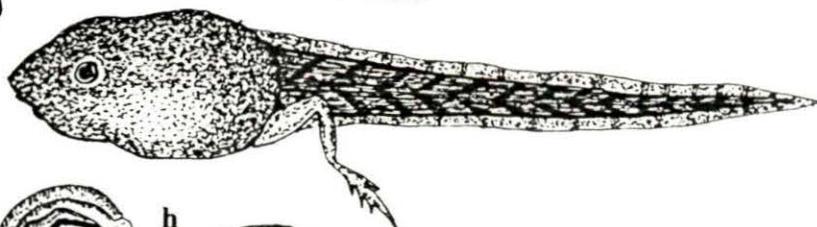
f



STAGE 35



g



STAGE 36



h



STAGE 37



i

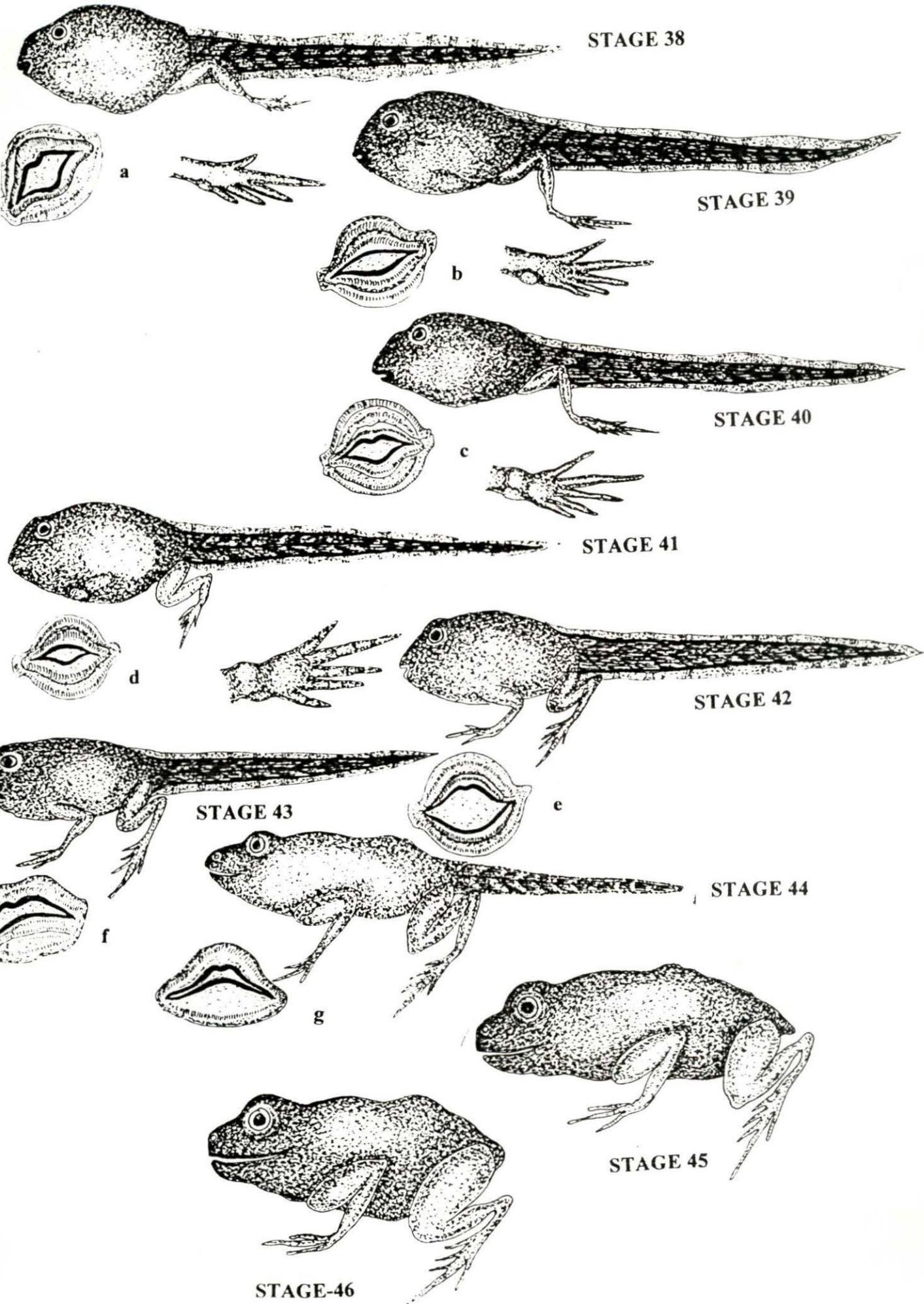


PLATE 4

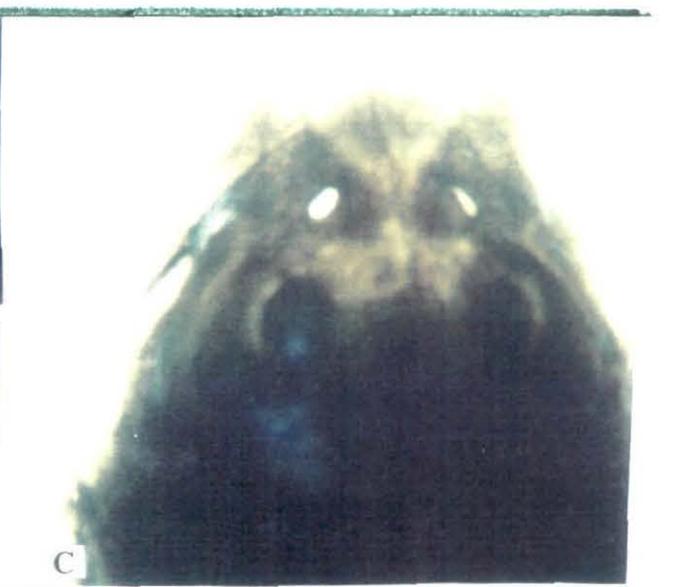
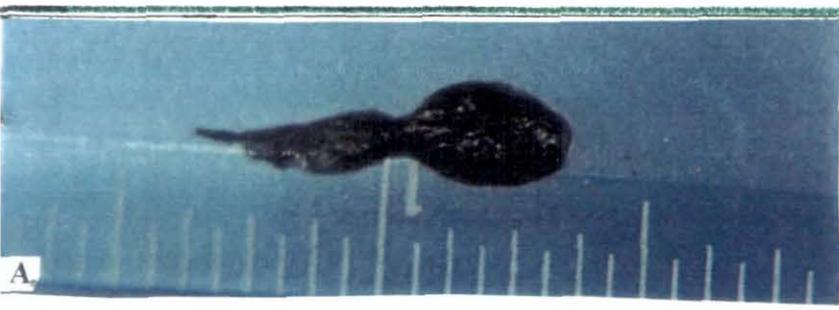
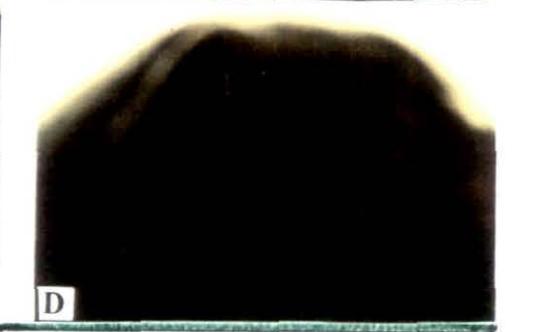
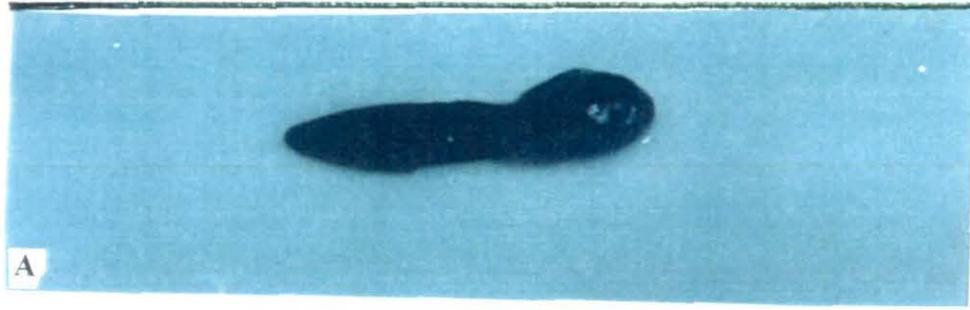
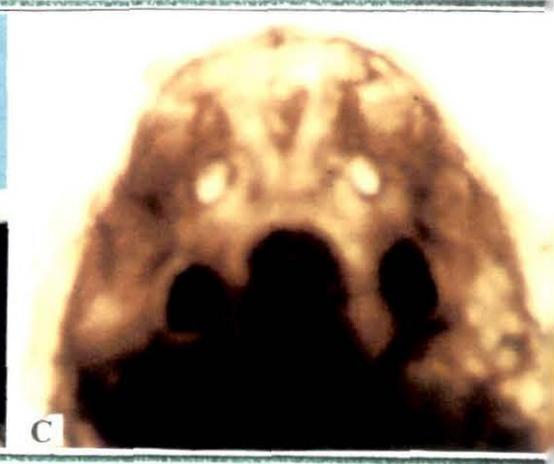
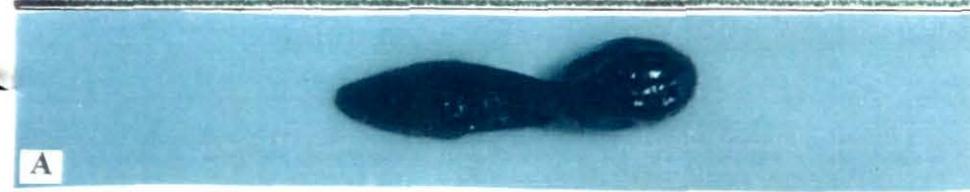
Legends

Normal life table of *Bufo himalayanas*

STAGE 20: A. Entire larva B. Tail portion of the larva enlarged
C. Dorsal view of the head enlarged

STAGE 21: A. Entire larva B. Tail portion of the larva enlarged
C. Dorsal view of the head D. Mouth disc of the larva

STAGE 22: A. Entire larva B. Tail portion of the larva enlarged
C. Dorsal view of the head



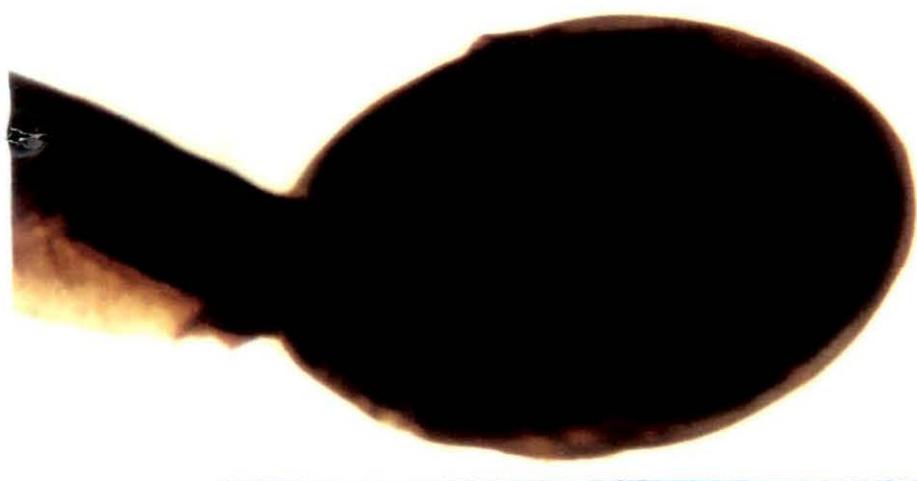
STAGE 22

PLATE 5

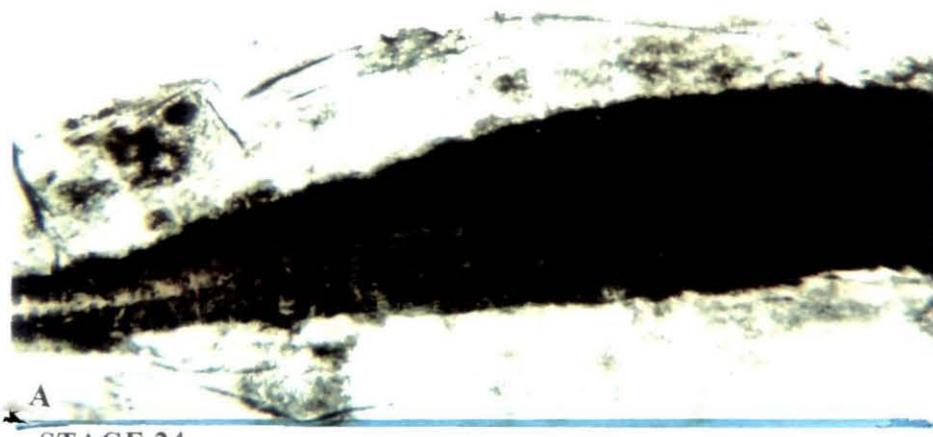
Legends

Normal life table of *Bufo himalayanas*

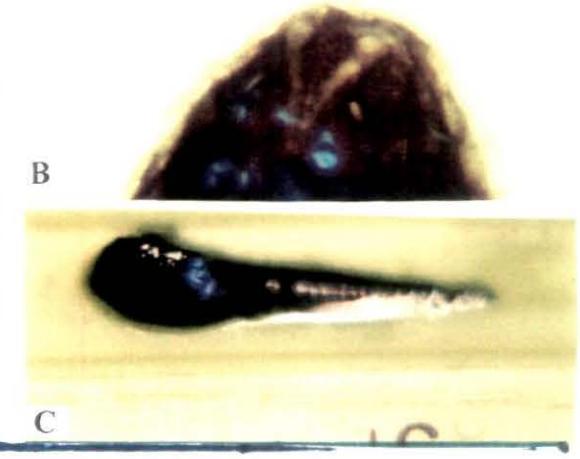
- STAGE 23: A. Head and portion of head enlarged
B. Dorsal view of the head C. Entire larva
- STAGE 24: A. Tail portion of the larva enlarged
B. Dorsal view of the head C. Entire larva
- STAGE 25: A. Junction of head and tail enlarged to show hind limb
bud B. Dorsal view of the head C. Entire larva
- STAGE 26: A. Junction of head and tail enlarged to show hind limb
bud B. Dorsal view of the head C. Entire larva



STAGE 23



STAGE 24



STAGE 25



STAGE 26



PLATE 6

Legends

Normal life table of *Bufo himalayanas*

- STAGE 27: A. Junction of head and tail enlarged to show hind limb bud B. Dorsal view of the head C. Entire larva
- STAGE 28: A. Junction of head and tail enlarged to show hind limb bud B. Dorsal view of the head C. Entire larva
- STAGE 29: A. Junction of head and tail enlarged to show hind limb bud B. Dorsal view of the head C. Entire larva
- STAGE 30: A. Junction of head and tail enlarged to show hind limb bud B. Dorsal view of the head C. Entire larva

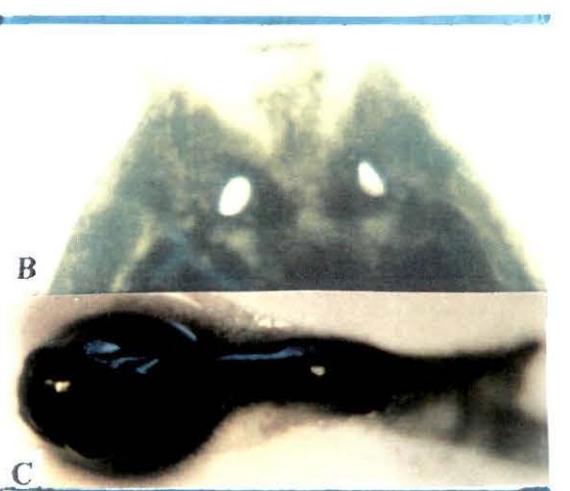
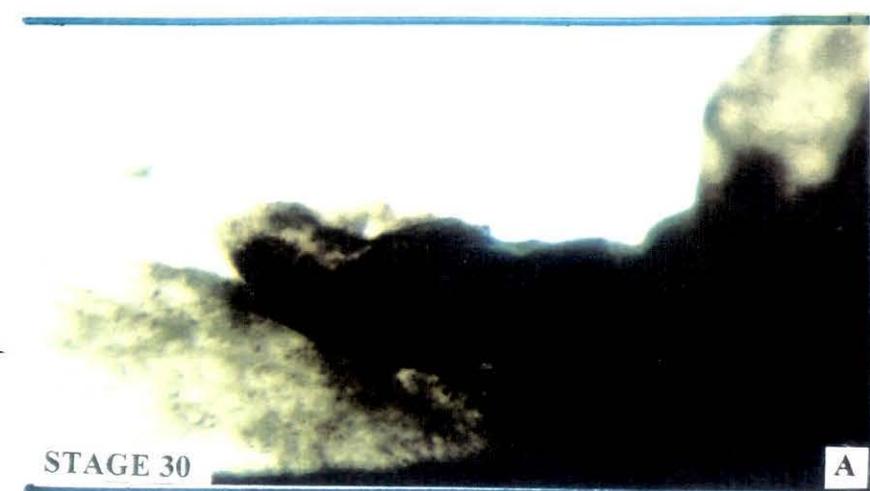


PLATE 7

Legends

Normal life table of *Bufo himalayanas*

- STAGE 31: A. Developing hind limb highly enlarged; dorsal view of the head (inset) B. Mouth disc enlarged C. Entire larva
- STAGE 32: A. Developing hind limb highly enlarged
B. Mouth disc enlarged C. Entire larva
- STAGE 33: A. Developing hind limb highly enlarged
B. Mouth disc enlarged C. Entire larva

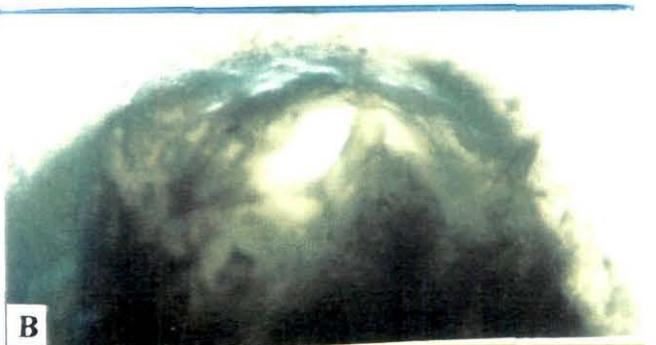
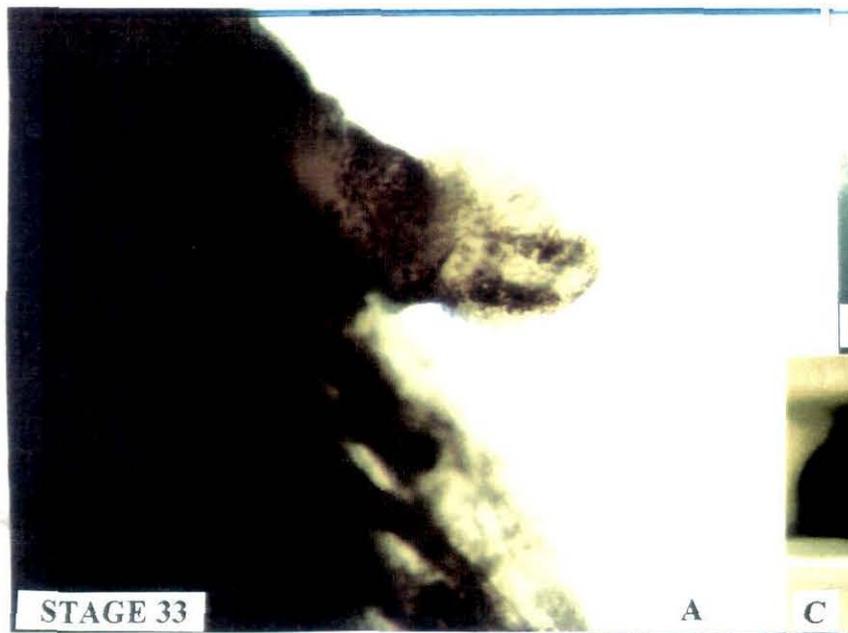
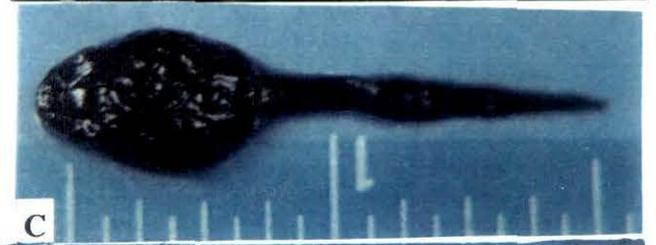
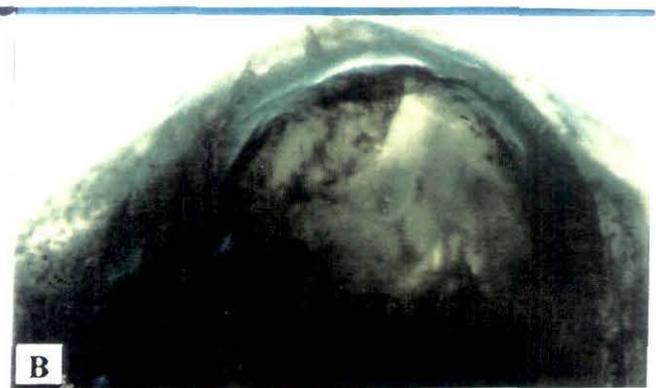


PLATE 8

Legends

Normal life table of *Bufo himalayanas*

- STAGE 34: A. Developing hind limb highly enlarged
 B. Mouth disc enlarged C. Entire larva
- STAGE 35: A. Developing hind limb highly enlarged
 B. Mouth disc enlarged C. Entire larva
- STAGE 36: A. Developing hind limb highly enlarged
 B. Mouth disc enlarged C. Entire larva

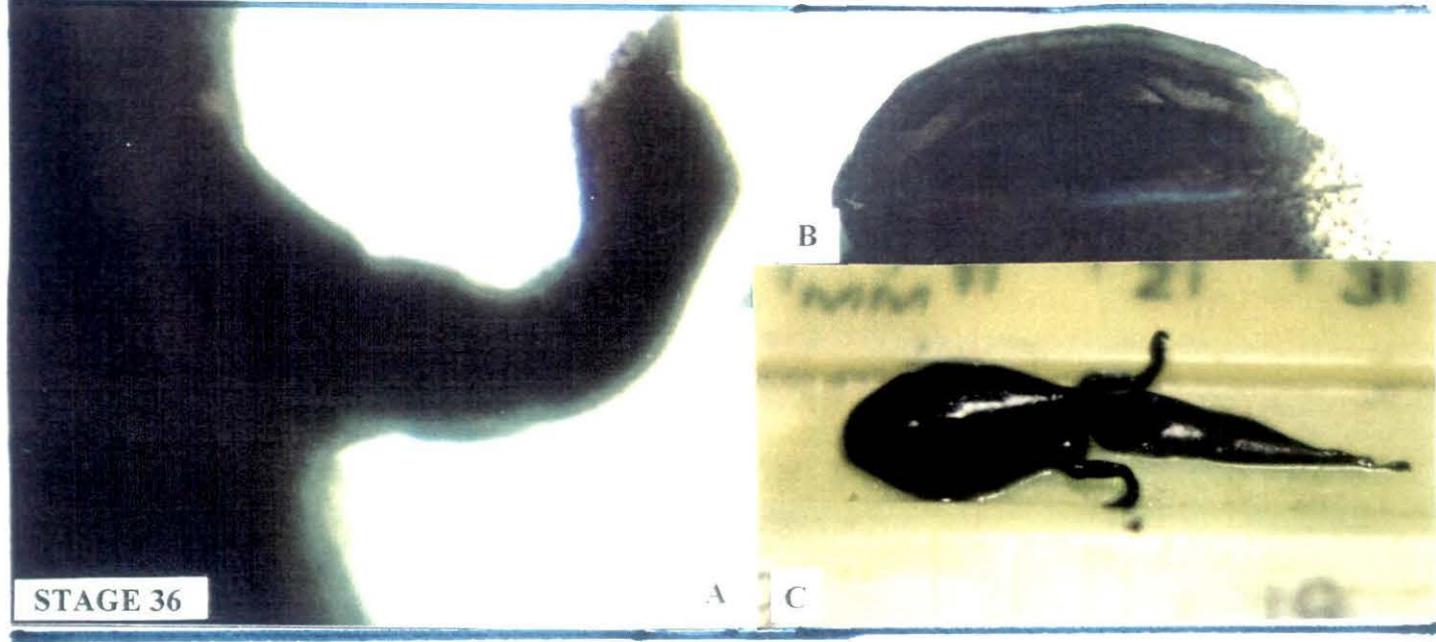
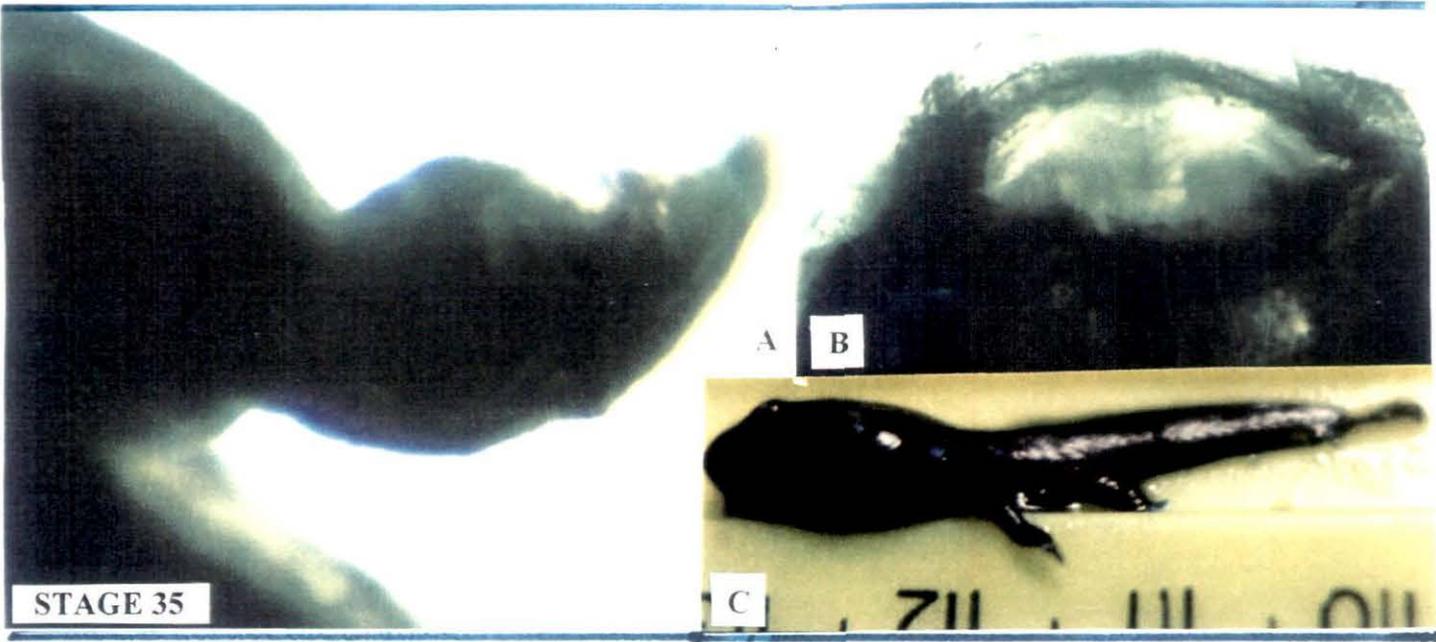
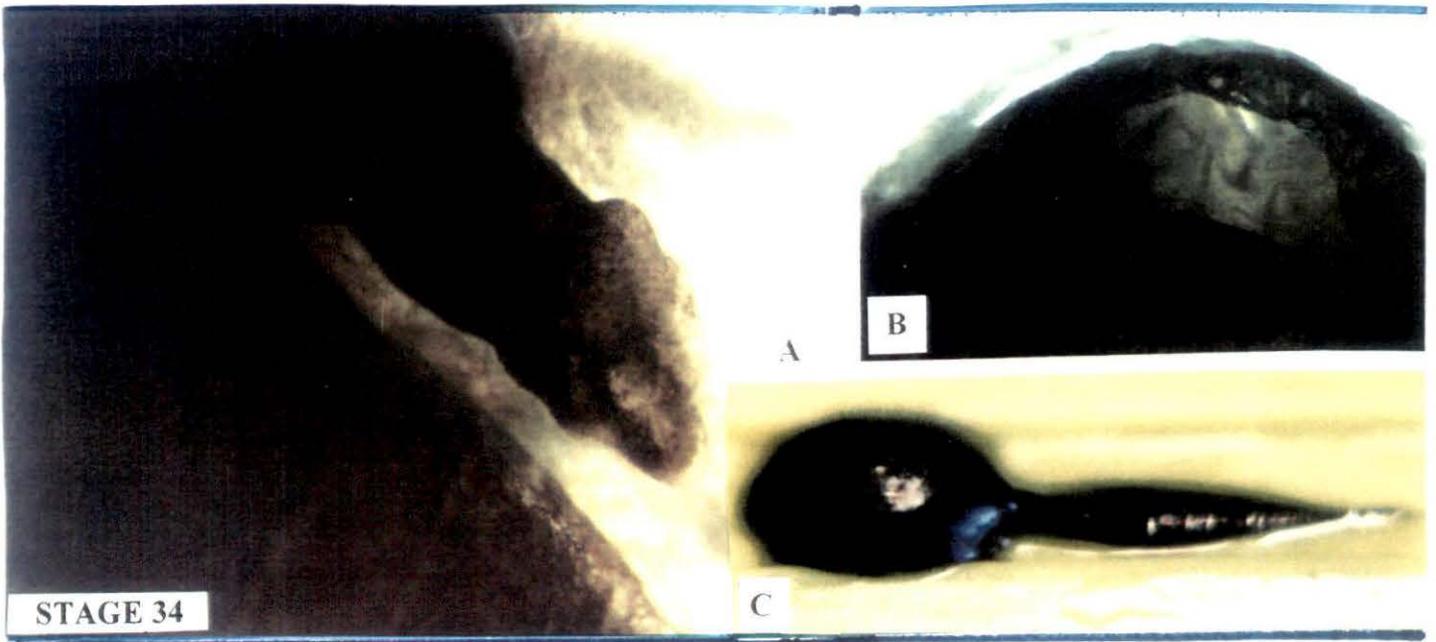


PLATE 9

Legends

Normal life table of *Bufo himalayanas*

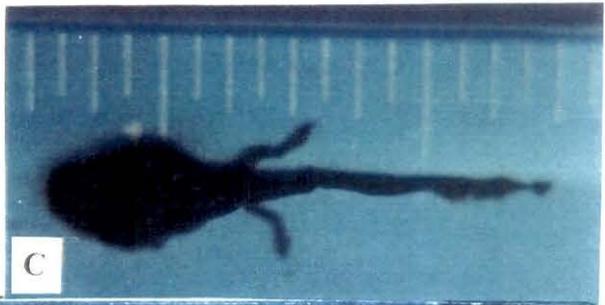
- STAGE 37: A. Developing hind limb enlarged to show digits
B. Mouth disc enlarged C. Entire larva
- STAGE 38: A. Developing hind limb enlarged to show digits
B. Mouth disc enlarged C. Entire larva
- STAGE 39: A. Toe of the hind limb highly enlarged to show complete
digitalization B. Mouth disc enlarged C. Entire larva



STAGE 37



B



A

C



STAGE 38



B



A

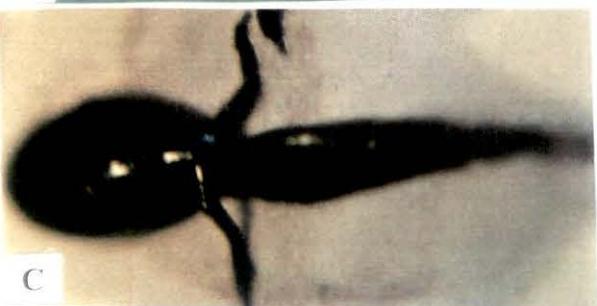
C



STAGE 39



B



A

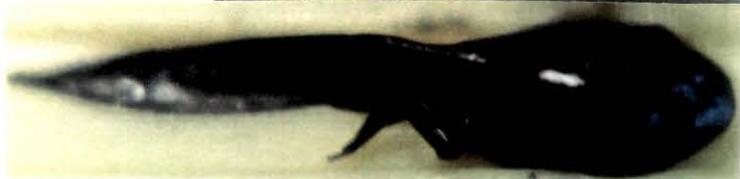
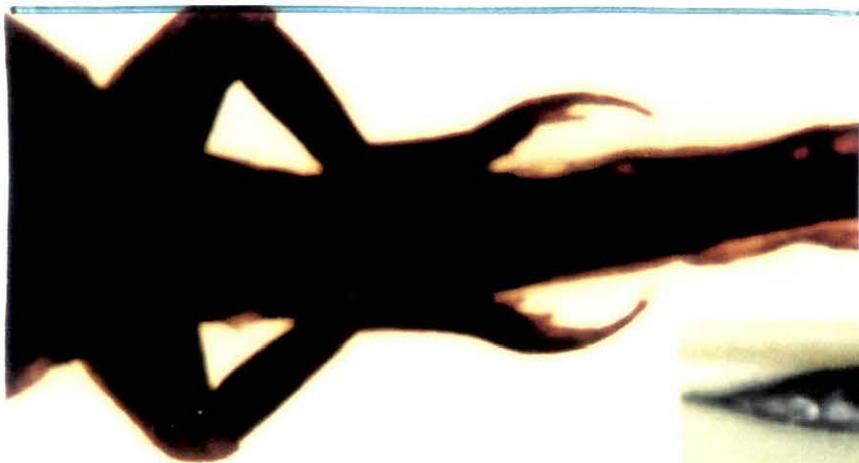
C

PLATE 10

Legends

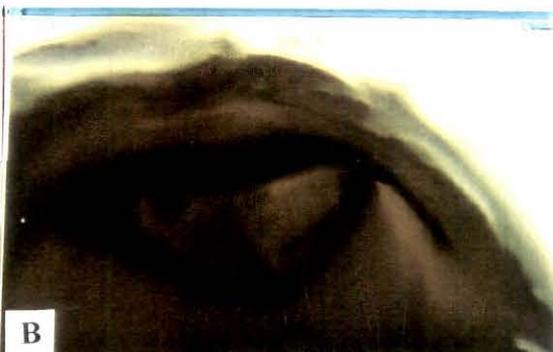
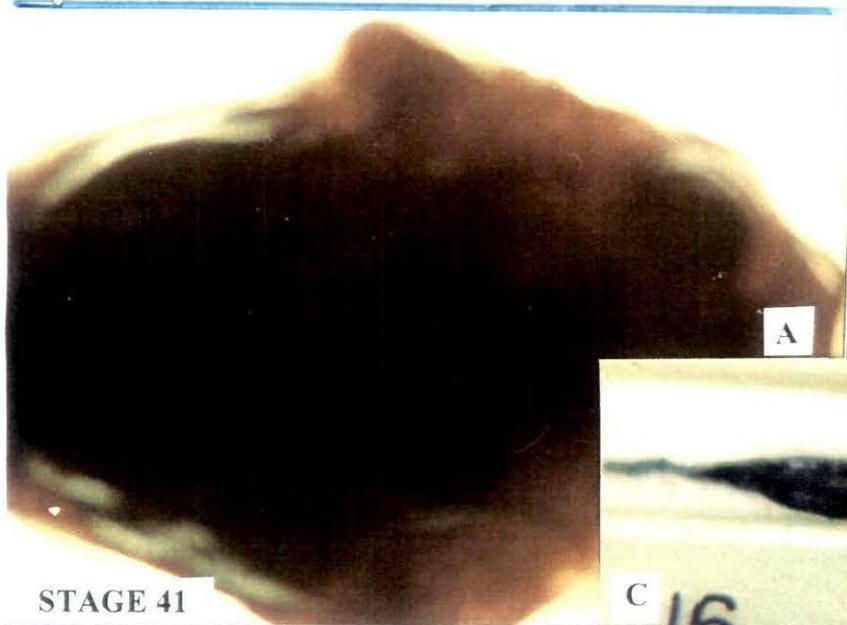
Normal life table of *Bufo himalayanas*

- STAGE 40: A. Both hind limbs along with tail enlarged
B. Mouth aperture enlarged C. Entire larva
- STAGE 41: A. head and body highly enlarged to show the forelimb
protrusion on the side of the head
B. Mouth aperture enlarged C. Entire larva
- STAGE 42: A. Forelimb protruded out with complete digitalization
B. Mouth aperture enlarged C. Entire larva



STAGE 40

A C



STAGE 41

A B

C



STAGE 42

A C

PLATE 11

Legends

Normal life table of *Bufo himalayanas*

- STAGE 43: A. Dorsal view with limbs and regressing tail
B. Mouth aperture enlarged C. Entire larva
- STAGE 44: A. Anterior part, dorsal view, head and body
B. Mouth aperture, ventral view C. Entire larva
- STAGE 45: A. Hind portion of the body showing tail knob
B. Head enlarged showing mouth and eyes.
C. Entire larva, tail almost totally regressed

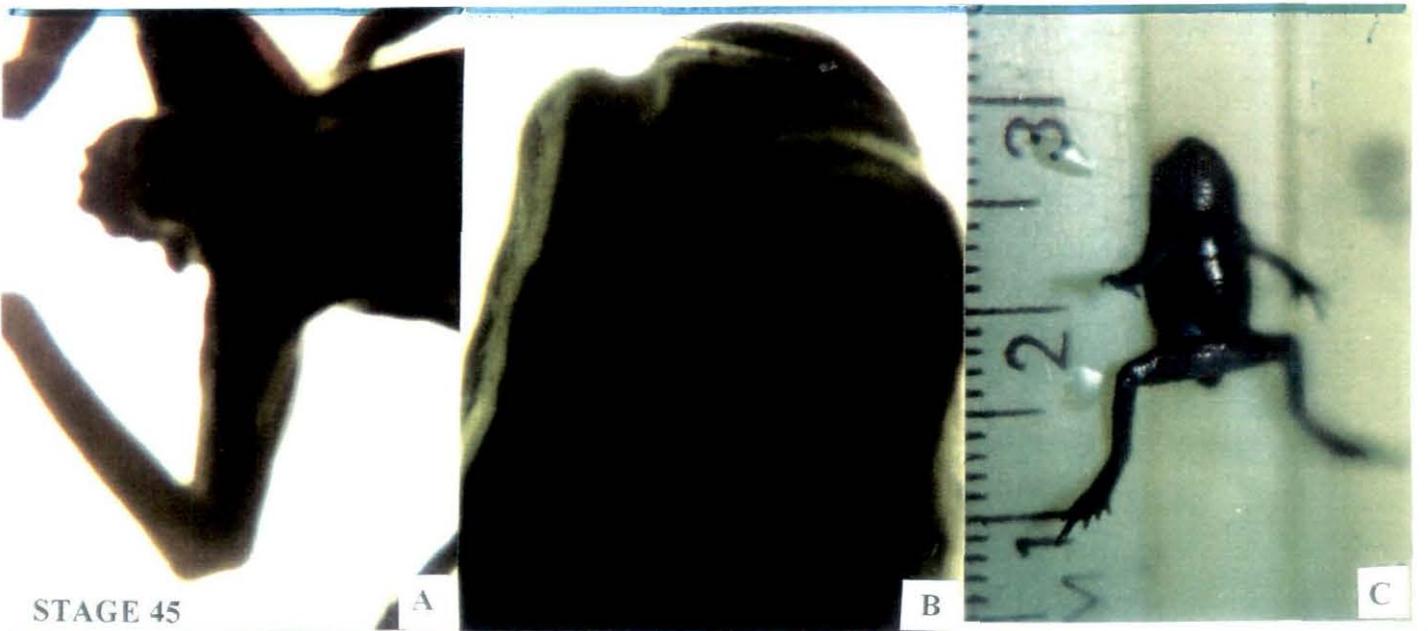
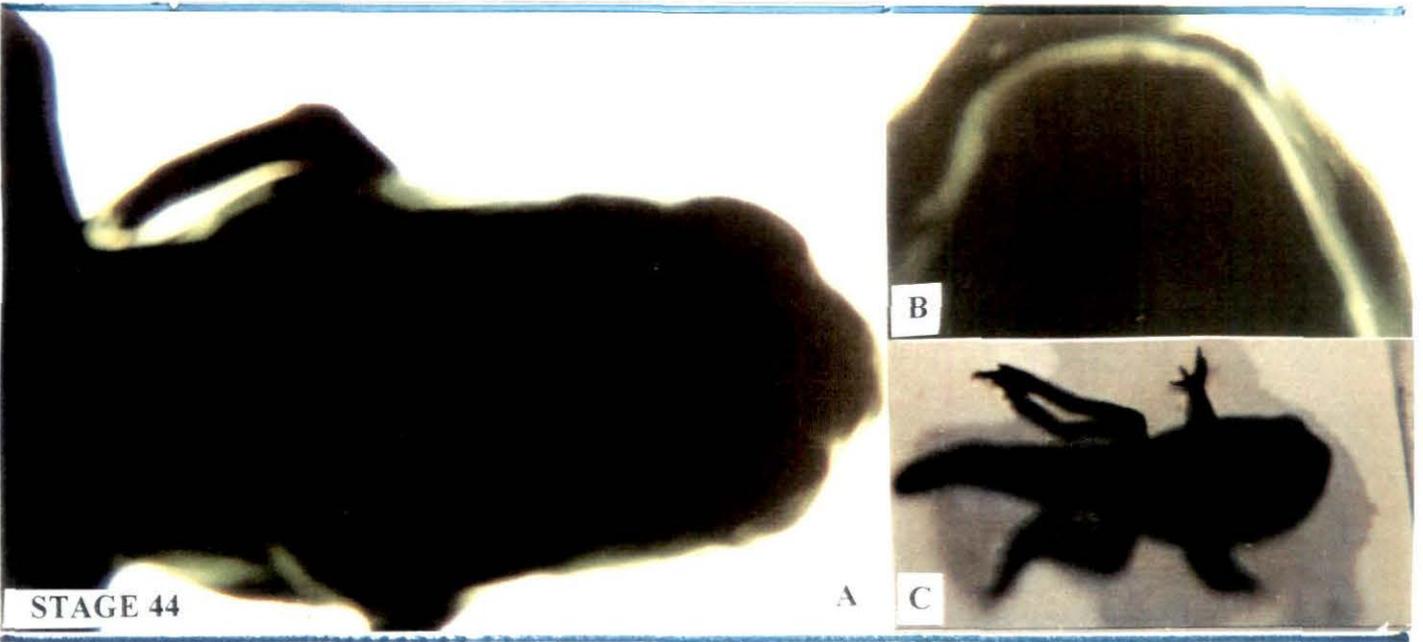
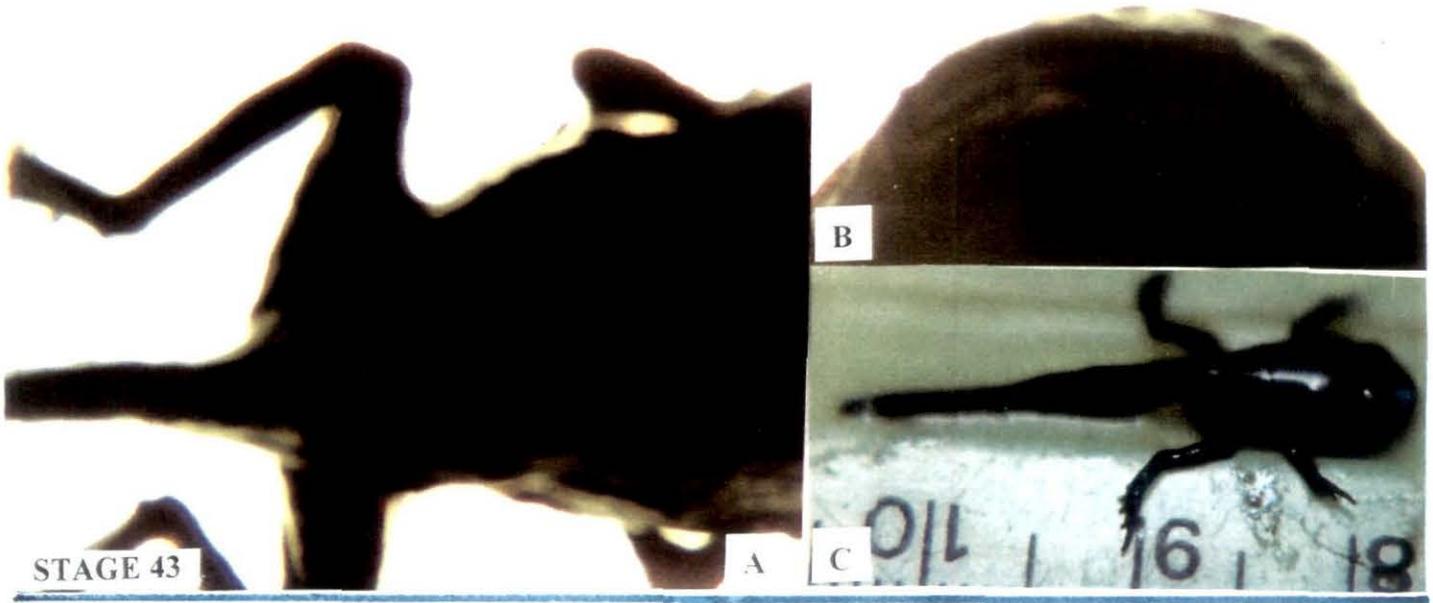
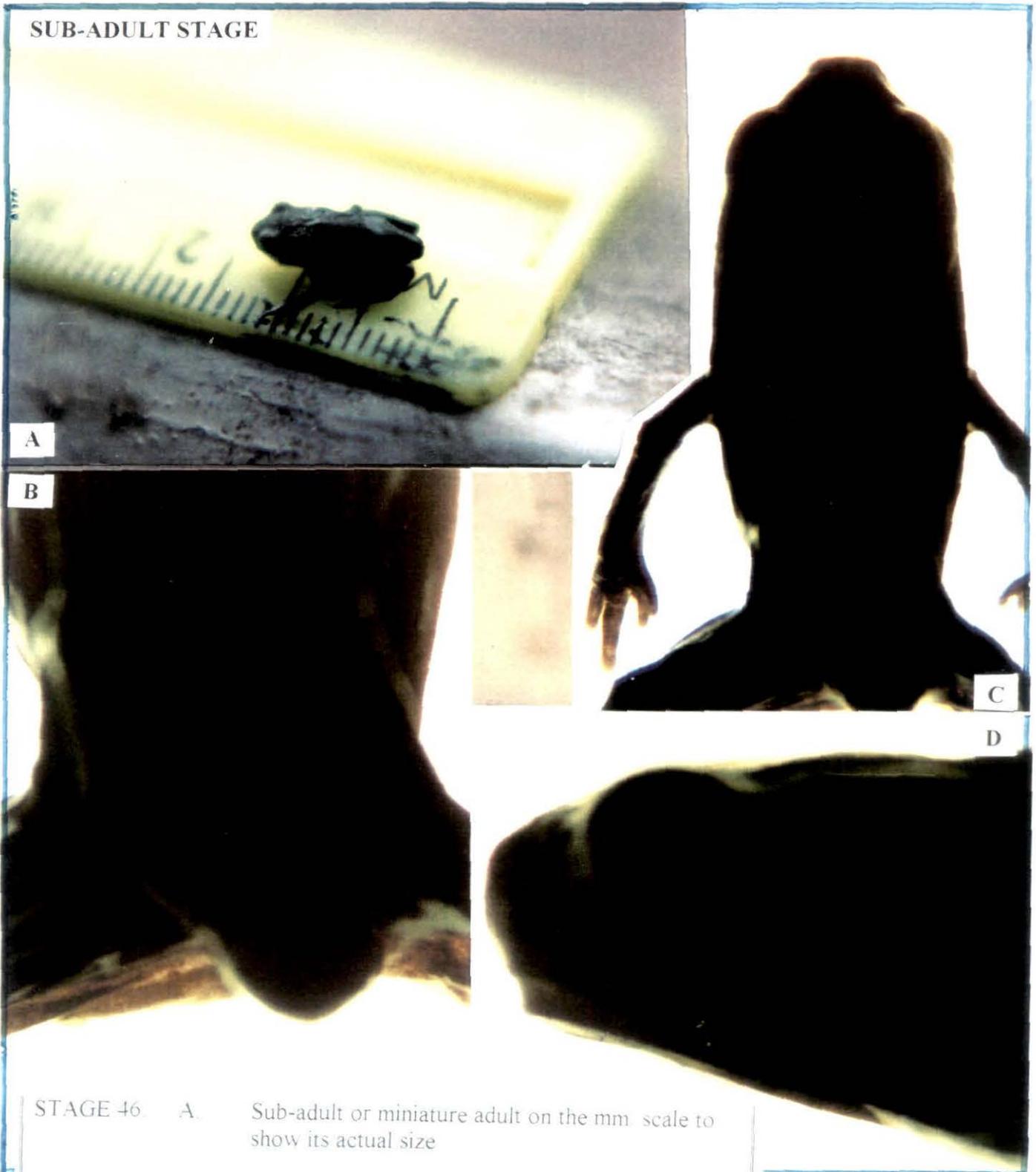


PLATE-12



- STAGE 46.
- A. Sub-adult or miniature adult on the mm. scale to show its actual size
 - B. Hind part of the body enlarged to show the fully regressed tail
 - C. Body with head and portion of the limbs

DISCUSSION

Metamorphosis in anurans involves the most comprehensive and most dramatic transformations of all major living chordate groups. This post-embryonic process systematically transforms most, if not all, organs of a tadpole to their adult forms. It also brings about the development of organs that only function in the adult stage. The changes that are found during metamorphosis can be resolved into three main categories:

(a) Resorption of tadpole specific organs:

The tadpole specific structures, such as tail, gills etc. are removed during metamorphosis. The two major tadpole organs, i.e., tail and gill, degenerate completely during this transition. Of the two, tail resorption has been extensively studied; but the gill degeneration has received little attention (Dodd and Dodd, 1976; Atkinson, 1981; Yoshizato, 1989).

The tail represents a simple but complex organ of larval population makeup of a diverse type of tissue, viz., epidermis, connective tissue, blood vessel, notochord and muscle. Despite such diversity in tissue types, all tail tissues are resorbed by the end of metamorphosis.

Two different processes appear to contribute to tail resorption. These are condensation and histolysis (Yoshizato, 1989). Condensation is an important factor contributing to the length reduction and is believed to be caused by water loss resulting in the compaction of cells and the extracellular matrix (Frieden, 1961; Lapiere and Gross, 1963; Yoshizato, 1989, 1996).

Histolysis in the tail has been studied in the skin and fin (Kerr et. al.,1974;Kinoshita et. al.,1985; Yoshizato,1989). It has been found that hitolysis starts at the outermost layers and propagates towards the inner layer.

In recent years the resorption of tail has been equated with the programmed cell death or apoptosis. Kerr et. al., (1972,1974) first demonstrated that in the tail resorption apototic like changes like nuclear and cellular fragmentation, formation of membrane bound vessicles (apototic bodies) containing cellular and cellular fragments, including condensed chromatin fragments etc. occur. These observations have later been supported by other workers, viz., Willy et.al., (1980); Kinoshita et. al.,(1985); Nishikawa and Hayashi (1995); Nishikawa et.al., (1998) and others.

In anurans the time and stage of tail resorption varies among species within a narrow range. In *Xenopus laevis*, the most noticeable early change in the tail degeneration occurs around stage 60 (Nieuwkopp and Faber,1956), around stage 60-61 in *Rana catesbiana* (Atkinson,1981).

Similarly the gill degeneration follows a similar fashion in anurans. Gill resorption begins around 61-62 stage in *Rana catesbiana* and by stage 64-66, complete resorption of gill takes place (Atkinson and Just,1975; Atkinson,1981). The total resorption of the gills occur relatively late, around the same stage or slightly earlier than tail resorption (Atkinson,1981) (Table-6).

(b) De novo development of organs

The development of limbs in an adult specific structure that develop during metamorphosis. Even though the limbs are made of similar tissue/ cell types as the tail, including skin muscle, connective tissue and cartilage, they develop progressively whereas the tail resorbs. Shi (2000) has stated that limb development is a thyroid hormone (TH)-dependent process while the tail resorption takes place when TH is present.

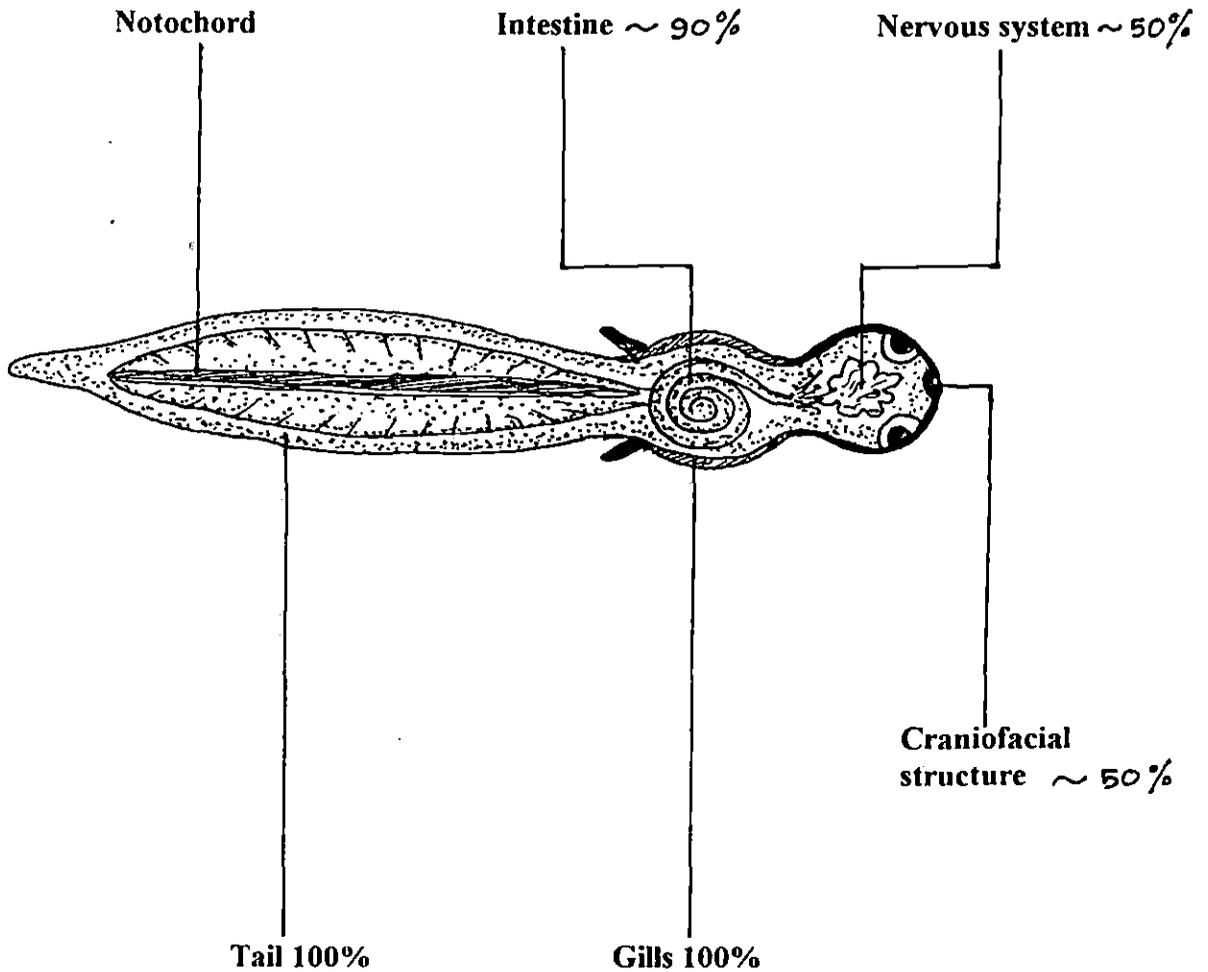
The requirement for TH in hind limb morphogenesis by experiments by manipulating the levels of TH in premetamorphic tadpole resulting faster morphogenesis of hind limb (Kattenbach,1953; Dodd and Dodd,1976) or inhibiting the synthesis of endogenous TH (Nieuwkoop and Faber, 1956).

(c) Remodelling of existing organ for adult use:

The majority of the organs are present in both tadpole and adult toad or frog. During metamorphosis, they undergo partial but profound transformations (Dodd and Dodd,1976; Gilbert and Frieden,1981; Fox,1983; Balls and Bownes,1985).

Several excellent reviews have described these processes representing different types of remodelling. For example, in case of liver remodeling the biochemical changes associated with the transition from ammonotelism to ureotelism thought to be due to reprogramming of the gene expression profiles of the existing hepatocytes into an adult like profile (Atkinson et. al., 1994,1996; Chen et.al.,1994,1998). Similarly remodeling in the nervous system (Dodd and Dodd,1976; Fox,1983; Gona et.al.,1988;Tata,1993) and that of the intestine(Glass,1968;

DIAGRAM : 5



Schematic representation of the major tissues of an anuran tadpole undergoing degeneration during metamorphosis. The numbers indicate the extent of programmed cell death expressed as percent of larval cells lost upon completion of metamorphosis

Segal and Petras,1992; Shi and Ishizuya-Oka,1996) and other organs have been encountered (Diagram 5).

Resorption of tadpole specific structures and remodeling of the existing organs for adult life form in recent years have been reviewed under a very specialised genetically controlled process called 'Programmed cell death'(PCD) or 'Apoptosis'.

Kerr et.al.,(1972) first through a series of microscopical observations found that cell death under physiological and pathological conditions occurs with distinct morphological changes, including membrane blebbing, chromatin condensation and cytoplasmic and nuclear fragmentation to form membrane enclosed vesicles containing chromatin fragments called 'apoptotic' bodies. Programmed cell death or apoptosis has now been recognized and unveiled in tail resorption (Kerr et.al.,1974; Wyllie et. al.,1980; Nishikawa and Hayashi,1995; Nishikawa,1998), intestinal remodeling (Smith-Gill and Carver,1981; Dauca and Hourdry,1985; Yashizato,1989; Shi nad Ishizyua-Oka,1996; Shi,1998, Shi,2000).

In this current investigation on larval development of *Bufo himalayanus* (Gunther), a himalayan high altitude toad, reveals that the developmental stages are similar with other anurans as well as urodel species which are found in high altitude as observed by Harrison (1969); Dasgupta (1988) and others.

From the normal table of development of this species it has been found that breeding season starts before the onset of winter within April to August. Developmental changes in larval morphology and their measurements of size tabulated in the format described by Gosner (1966). From this normal table it is

observed that after hatching the Stage-20 emerge and upto Stage -25 only the length is increased, no special morphological changes observed except the formation and development of larval mouth.

At stage 26, hind limb bud appears. Hind limb shows prominent appearance and develops faster at stage 29. It is important to note that from stage 29 to stage 40 no morphological change happens except the limb development or digitalisation. This observation has similarities with other species of *Bufo* (Limbaugh and Volpe, 1957). The unique phenomenon of metamorphosis or drastic morphological changes occurred during developmental stage 41 to stage 46. This characteristic is also common with other anuran species. A comparison of larval anuran stages (Just et al., 1981) has been presented here in Table -6. Here the developmental sequences have a keen relationship with environmental temperature. This high altitude species thus shows its normal course of development at laboratory conditions where temperature is always kept below 20 °C to 22 °C. Studies in their habitat will establish the normal duration of the larval phase of the species especially, which will ensure non-interference of laboratory factors that may influence the result to some extent.

TABLE- 6

COMPARISON OF LARVAL ANURAN STAGES (Just et.al., 1981)

STAGES																								
SPECIES	LIMB BUD GROWTH				TOE DIFFERENTIATION				RAPID HIND LIMB GROWTH				TAIL RESORPTION											
<i>Bufo bufo</i> (Rossi,1959)	23	24	25	I	II	III	IV	V	VI	VII	VIII	VIII	IX	X	X	X	XI	XI	XII	XIII	XIV	XIV	XV	
<i>Rana pipiens</i> XXIV XXV (Taylor and Kollros,1946)	I	II	III	IV	V	VI	VII	VII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX	XX	XXI	XXII	XXIII	
<i>Xenopus laevis</i> (Nieuwkoop and Faber,1956)	46	47/48	49/50	51	52	53	53	54	55	55	56	57	57	57	58	59	60	60	61	62	63	64	65	66
	I-----I				I-----I				I-----I				I-----I											
	PREMETAMORPHOSIS				PROMETAMORPHOSIS								CLIMAX											
Metamorphic Subdivisions (Dodd and Dodd, 1976)																								