Chapter III

Development of *Rana alticola*
INTRODUCTION

The development of the frog can be divided into two stages, a larval stage and an adult stage. The process by which the larval tadpole transforms into the adult frog is referred to as metamorphosis. During the larval period, amphibians, anurans in particular, exhibit a series of dramatic morphological changes (e.g., tail formation, perforation and closure of the spiracle, limb formation, tail reduction). Metamorphosis is a post-embryonic period of profound morphological changes by which the animal alters its mode of living. Anuran metamorphosis is separated into three specific periods - pre-metamorphosis, pro-metamorphosis and metamorphic climax (Etkin, 1964; 1968; Dodd and Dodd 1976). Metamorphosis in anurans involves resorption of the tail, development of the front and hind limbs and large changes in most organ systems. Not only do different organs undergo different changes, but they also occur at distinct developmental stages to coordinate the effective transition of a tadpole to a frog (Shi, 2000).

The tadpoles of anuran amphibians are the seemingly odd organism with a composite head and body, a muscular tail without vertebrae and dorsal and ventral fins that lack bony supports. They possess a pair of eyes and usually external nares. A spiracle(s) that provides an exit for water pumped through the respiratory and food-trapping structures may occur in assorted positions in different species. The intestine is generally long and arranged in a spiral manner. The liver is large. These two organs are the major components of the viscera and often visible from the ventral side. A structurally variable and evolutionarily unique oral apparatus typically composed of soft and keratinized parts facilitates the harvesting of a myriad of food sources. Numerous morphological variations encountered not only reflect
adaptations to diverse habitats such as puddles, ponds, stagnant or gently flowing water bodies and even fast flowing streams but also phylogeny (Duellman and Trueb 1986, McDairmid and Altig 1999).

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 (Duellman and True, 1994; Altig and McDiarmid, 1999). Most amphibians have aquatic pre metamorphic life stages consisting of a relatively short (hours to days) embryonic period followed by a substantially longer larval period, lasting from days to years, depending on species. The duration of the larval period thus puts amphibians at risk of chronic exposure to contaminants in water and sediment. Not only are larval amphibians faced with potential chronic abiotic stresses, but biotic stress can also be quite severe. The larval period is a time of rapid growth and subsequently high nutritional requirements, which can lead to severe inter-and intra specific competition, even in systems that support a healthy resource base (Brockelman 1969; Alford and Wilbur 1985). Recruitment of juveniles must therefore reflect an interaction among abiotic and biotic stresses incurred during the embryonic and larval life stages (Dunson and Travis 1991). Such an interaction may be important in contaminated breeding habitats; contaminants may affect larval amphibians directly, via influences on physiological, behavioural, and morphological traits (Rowe et al, 1996, 1998a, 1998b; Raimondo et al, 1998; Hopkins et al, 2000), and indirectly via influences on resource abundance, thereby affecting competition.
Amphibian development has been investigated extensively by embryologists, who have taken advantage of the development of relatively large external eggs for both descriptive and experimental studies. As biology emerged as a science in the late 1600s and early 1700s; amphibians played an important role in research. For example, the first description of cleavage in a zygote was of a frog egg by Swammerdam (1738). Since the beginning of the 20th Century, the need for normal table of anuran development has been felt. Adler (1901) gave the earliest developmental table where he divided the entire developmental period of *Bufo regularis* into 15 stages. Pollister and Moore (1937) divided the developmental period of *Rana sylvatica* up to limb bud stage into 25 stages. Shumway (1940) divided the developmental period of *Rana pipiens* up to complete operculum stage into 25 stages. Taylor and Kollros (1946) studied the post embryonic development of *Rana pipiens* from Shumway stage 25 and divided the period of metamorphosis into another 25 stages. Anuran development is described thoroughly by Rugh (1951), who also treated experimental embryological work on amphibians in detail (Rugh, 1962). Complete tables of development are necessary for accurate comparison of developmental stages in different organisms (Duellman and Trueb, 1994). Many different methods have been developed to stage anurans during development, especially during metamorphosis (Just et al., 1981). After this, many workers concentrated on providing developmental stages of different anuran species. Since the description of developmental table involved two systems and lot of variation was found in the developmental patterns of different species, Gosner (1960) reviewed the problem and provided a simplified table for staging anuran embryos and larvae.
Amphibian development has been investigated extensively by many embryologists, but the most comprehensive treatment of amphibian development within a broad biological context is the work of Salthe and Mecham (1974). Complete tables of development are necessary for accurate comparison of developmental stages in different organisms (Duellman and Trueb, 1994). Many different methods have been developed to stage anurans during development, especially during metamorphosis (Just et al., 1981). The most commonly used staging method for Rana pipiens and Rana catesbeiana is that of Taylor and Kollros (1946) whereas that for Xenopus laevis is that of Nieuwkoop and Faber (1956). Various authors like Dutta and Mohanty-Hejmadi, 1976; Agarwal and Niazi, 1977; Roy and Khare, 1978, Mohanty-Hejmadi et al., 1979 a, b, 1980; Kiyasetuo and Khare, 1986b; Dutta et al., 1990-91; Patil and Kanamadi, 1997; Ao and Bordoloi, 2001; Dutta et al., 2001) have contributed to the study of normal table of development of anurans in India.

Studies have been conducted on the life history and metamorphosis of some ranids from India. Although Rao (1915) and Ferguson (1964) have described a few larvae of the Indian burrowing frog Rana breviceps, there is considerable disagreement between the two regarding the characteristics. However, Mohanty-Hejmadi et al., (1979a) studied the life history including detailed characteristics of the larva and its teeth structure of the Indian burrowing frog Rana breviceps by raising eggs through metamorphosis. Few reports are available on the normal tables of development of ranids, Rana (Hoplobatrachus) tigerina (Annandale, 1912; Annandale and Rao, 1918; McCann, 1932; Kirtisinghe, 1957; Bhati, 1969; Dutta and Mohanty-Hejmadi, 1976; Agarwal and Niazi, 1977), Rana (Fejervarya) limnocharis

Tadpole survival depends on many physical, chemical and biological factors. Mortality during the early developmental stages is high in amphibians, mainly due to predation by invertebrates and vertebrates. In general, survivorship of ranid larvae has been estimated to be around 5%. In microhabitats such as tree holes or leaf axils, food is limited and cannibalistic behavior has also developed as a means for survival. The growth rates of anuran tadpoles are known to depend upon various factors such as density and inter and intra-specific competition for food and space and other resources (Dash and Hota 1980, Hota and Dash 1981, Hokit and Blaustein 1997, Girish and Saidapur 1999, Saidapur and Girish 2001). The duration of different stages of larval development vary with the species and also depending upon various factors such as temperature, food availability, aeration, crowding and kinship. Consequently, the duration of metamorphosis also varies. In general those that exhibit a rapid development metamorphose early and emerge as adult morphotypes at a smaller size while those having a prolonged larval life emerge as froglets at a larger size. Larger tadpoles swim faster (Wassersug and Hoff 1985) and may be more capable of escaping predators (Feder 1983). Risk of predation is thus often size-specific, either decreasing monotonically with increasing tadpole body size (Richards and Bull 1990a, b) or increasing to a maximum and then decreasing (Brodie and Formanowicz 1983; Wilbur 1988). Tadpoles may also alter predation risk by behaving differently when predators are present. Behavioral changes often involve alterations in microhabitat selection (Formanowicz and Bobka 1989; Hews 1995; Horat and Semlitsch 1994; Petranka *et al.*, 1987; Semlitsch and Reyer 1992)
or total activity level (Chovanec 1992; Hews 1988; Lawler 1989; Skelly and Werner 1990).

Time to metamorphosis and rate of development for anuran tadpoles is influenced by the temperature (Harkey & Semlitsch, 1988), size (Pearman, 1993) and rate of dessication (Newman, 1989) of their habitat (Denver, 1997), plus density (Gromko et al., 1973, Miranda & Pisano, 1993), pathogens (Beebee, 1995b; Petranka, 1995) and diet (Kupferberg, 1997a). During development, the embryonic stage appears to be most sensitive: low pH leads to a denaturation of the hatching enzyme (Urch and Hedrick, 1981) and subsequently to deformations of the embryos and high embryonic mortality. The effects of environmental pH on growth and development of tadpoles are of interest because of the natural acidity of some wetlands (Gosner and Blake 1957), and because acidification by human activities may have a major impact on frog populations (Henrickson 1990). Changes in environmental acidity may also affect the interactions of species with their predators, sometimes reducing the vulnerability of tadpoles to size-limited predators by decreasing predator growth rates, as Kiesecker (1996) demonstrated for the interaction between larvae of *Pseudacris triseriata* and *Ambystoma tigrinum*. Responses to increased acidity can depend on larval size or developmental stage (Rosenberg and Pierce 1995) so that the effects of transient increases may depend on their timing relative to the larval life span of affected species.

In general, the morphological variations are ecologically correlated. For instance, habitat selection and body form, foraging behavior and oral armature are interrelated. Lentic forms have weak tail musculature than the lotic forms, and the smallest muscles are associated with the largest fins (McDairmid and Altig 1999).
The benthic forms typically possess a dorsoventrally flattened body, dorsal eyes and low fins whether a lentic or lotic environment. On the other hand, lentic (pond), nektonic (rarely lotic) forms may have compressed, depressed or equidimensional bodies and they live in different parts of water column. A surface dweller, on the other hand, will typically have a laterally compressed body and a well developed ventral fin. The burrowing forms and those that live in confined spaces are vermiform with depressed bodies, dorsal eyes and low fins. Semi terrestrial tadpoles have elongate bodies, narrow tail muscles with abbreviated fins, large eyes that bulge above the surrounding body surface and hind limbs that develop precociously (McDairmid and Altig 1999).
MATERIALS AND METHODS

To study the development of *Rana alticola* three study sites namely Sairang River (Study site I), Herhse stream (Study site II) and Tamdil Lake (Study site III) were selected. Although breeding activities start from late June, it is to be mentioned that the developmental time in the natural condition (field) was studied from September to April (2005 to 2007) when the level of the water has subsided and the current of the water has also slowed down making it possible to make an enclosure in the field for the study of the development (Fig 3.47a and b).

Study of the development of *Rana alticola* was done at the study sites I (Sairang River), II (Herhse Stream) and III (Tamdil Lake). The amplexing pairs were brought to the laboratory and allowed to lay eggs in the laboratory condition. The numbers of eggs were then counted in order to know the clutch size. The time of development was observed under a stereoscopic dissecting binocular microscope. The time of onset of each new stage was noted and each developmental stage was fixed in a mixture of 70% alcohol and 4% formaldehyde in the ratio of 1:1. Staging of the developmental stages was done on the basis of external morphological changes as per the criteria described by Gosner in 1960. The different developmental stages were photographed with the help of microscope (Magnus MLX) with photographic attachments. The eggs were allowed to develop in a plastic tray fed with water from the study sites. As development progressed and the embryo hatched and started feeding, they were fed with algae collected from the study sites. The water temperature in the laboratory was also maintained as in the field as far as possible. Simultaneous observation was done in the field and laboratory during the study periods. During the early part of development, hourly observation was done in
the field itself where an enclosure was made with the help of net at the study sites and as development progressed, field was visited at regular interval till metamorphosis.

Throughout the study periods from 2005 to 2007, ecological factors like rainfall, relative humidity, air and water temperature, and pH were recorded at a weekly interval. Rainfall data was obtained from The Department of Agriculture and Minor Irrigation, Mizoram. Relative humidity was recorded with the help of hygrometer; temperature was recorded with the help of a thermometer, and pH with the help of a pH pen.
RESULTS

The present study describes the different developmental stages of *Rana alticola* following Gosner (1960). During the present study, it was recorded that the amplexing pairs oviposits in the laboratory condition and the time of oviposition was taken as the time of fertilization. The eggs were carefully observed under a stereoscopic dissecting binocular microscope and the time of onset of each new stage was noted and recorded. Simultaneously, eggs oviposited in the field were also observed in the natural condition where an enclosure was made with the help of a net (Fig 3.47 a and b). The eggs in the laboratory were maintained at water temperature between 11°C to 26°C which is more or less the temperature in its natural habitat which range from 10°C - 25°C from September 2005 to April 2006 and September 2006 to April 2007 at the three study sites (Table 2.4, Fig 3.57, 3.58, 3.59). Although breeding activities starts from late June, the developmental time in the natural condition (field) was studied from September 2005 to April 2006 and September 2006 to April 2007 during which the level of the water has subsided and the current has also slowed down making it possible to make an enclosure in the field for the study of the development. The time mentioned within parenthesis indicates the hours/ days of development taken from the time of fertilization. The developmental time is also shown in table 3.1.

**Stage 1 – Fertilization (0 hour):** The amplexing pairs were brought to the laboratory and the time of oviposition was taken as the time of fertilization and is indicated as 0 hour. The fertilized egg is spherical in shape. The egg is light brown in colour and pigmented at the animal half while the vegetal half is creamy white. Fertilization is
indicated by rotation of the embryo until the animal pole is uppermost. The eggs at
the time of laying measures between 1.2 mm to 1.5 mm in diameter (Fig 3.1).

**Stage 2** – Gray Crescent (30 min): After 30 minutes from the time of fertilization,
the gray crescent was observed. At this stage, a lightening gray crescent appears on
the portion of the pigmented area. The size of the egg remains the same (Fig 3.2).

**Stage 3** – Two cell stage (5 hrs): After 5 hours from the time of fertilization, the first
cleavage starts. The first cleavage plane is meridional and this cleavage furrow first
appears near the animal pole and progressively extends towards the vegetal pole of
the egg resulting in two equal blastomeres. It takes 1 hour 30 minutes to complete
the first cleavage. The size of the egg remains the same (Fig 3.3).

**Stage 4** – Four cell stage (6 hrs 30 min): When the first cleavage is about to
complete, the second cleavage furrow starts at the animal pole and it is holoblastic
and equal. The cleavage plane is meridional and at right angle to the first plane
dividing the egg into 4 equal parts. It takes 1 hour and 41 minutes to complete the
second cleavage. The size of the egg remains the same (Fig 3.4).

**Stage 5** – Eight cell stage (8 hrs 11 min): The third cleavage is horizontal and occurs
a little above the equator and at right angle to the first and second cleavages. This
cleavage results in the formation of four micromeres and four macromeres. The
micromeres are darker in colour than the macromeres. It takes 2 hours 14 minutes to
complete this stage. The size of the egg remains the same (Fig 3.5).

**Stage 6** – Sixteen cell stage (10 hrs 25 min): The fourth sets of cleavage planes are
vertical and holoblastic. It starts from the animal pole and the furrow progress
downwards dividing the egg into 16 blastomeres. It takes 2 hours 8 minutes to
complete the cleavage. The size of the egg remains the same (Fig 3.6).
Stage 7 – Thirty two cell stage (12 hrs 33 min): The fifth cleavage results in the formation of 32 blastomeres. Observation of the pattern of cleavage from 32 cell stage onwards is difficult and the cleavage furrow is irregular. The cells which are in the animal hemisphere are smaller than the cells at the vegetal hemisphere. It takes 2 hours 17 minutes to complete this stage. The size of the egg remains the same (Fig 3.7).

Stage 8 – Mid cleavage (14 hrs 50 min): This stage is characterized by continued irregular cleavage and there is intrusion of dark brown pigmented blastomeres over the light creamy blastomeres. The size of the egg remains the same (Fig 3.8).

Stage 9 – Late cleavage (16 hrs 50 min): Late cleavage was observed after 16 hrs from the time of fertilization. At this stage, the cells appear like a ball containing many smaller blastomeres. Some of the blastomeres appear brownish and some light creamy in colour. The size of the egg remains the same (Fig 3.9).

Stage 10 – Dorsal lip (44 hrs): There is invagination of the cell at dorsal lip of blastopore indicating the beginning of gastrulation. The size of the egg remains the same (Fig 3.10).

Stage 11 – Mid gastrula (48 hrs 25 min): Dorsal lip of blastopore expands into semicircle. The invaginated blastomeres which are now exposed within a ring form the yolk plug. The egg starts to change in shape from this stage and measures up to 1.6 mm (Fig 3.11).

Stage 12 – Late gastrula (58 hrs 35 min): At this stage the blastomeres invaginated inside the embryo through the blastopore and the yolk plug becomes smaller in size. The size of the egg remains the same as in the previous stage (Fig 3.12).
Stage 13 – Neural plate (4 days): About four days from the time of fertilization, the small protruding plug of yolk cells gradually disappears, and the neural plate develops as a tubular area on the dorsal surface. The shape of the embryo change and become slightly elongated and the dorsal surface is flattened to form the neural plate. The embryo measures 1.75 mm (Fig 3.13).

Stage 14 – Neural fold (5 days): Neural fold is marked by elongation of the embryo and the elevation of two lateral ridges separated by the neural grooves. Neural folds form as ridges lateral to the neural grooves. It takes 34 hours to complete this stage. The embryo measures 1.8 mm (Fig 3.14).

Stage 15 – Rotation (6 days 10 hrs): The neural folds approach each other closer and the neural grooves narrowed. The embryo begins to rotate and elongate. It takes 27 hours to complete this stage. The embryo at this stage measures 1.8 mm (Fig 3.15).

Stage 16 – Neural tube (7 days 20 hrs): At this stage, the neural folds are closed forming a neural tube. The gill plates become distinct and there is elongation of the embryo. It takes almost 2 days to complete this stage. The embryo measures 2 mm (Fig 3.16).

Stage 17 – Tail bud (9 days): Tail bud appears at the posterior end of the embryo. At this stage, a film of jelly like covering is formed within the existing jelly cover. The embryo begins to develop a recognizable head. It takes 3 days to complete this stage. The embryo measures 2.1 mm (Fig 3.17).

Stage 18 – Muscular response (12 days): The embryo shows muscular response to external stimuli. The embryo is elongated and the gill plates are observed. It takes almost two days to complete this stage. The embryo measures 2.3mm to 2.5 mm (Fig 3.18).
Stage 19 – Heart beat (13 days 20 hrs): The embryo hatched at this stage. Tail greatly elongated and pulsation of the heart is visible. External gill buds conspicuous. It takes 43 days to complete this stage. The hatchling measures 3.5 mm to 3.6 mm (Fig 3.19).

Stage 20 – Gill circulation (16 days 15 hrs): Opercular fold covered the base of the gills and oral sucker became well developed. The gills are visible from the outside and start to circulate. There is also elongation of the tail. It takes almost 2 days to complete this stage. The hatchling measures 4 mm (Fig 3.20).

Stage 21 – Cornea transparent (18 days 10 hrs): Cornea becomes transparent and oral suckers and nasal pits became prominent. The mouth starts to open at this stage. It takes about 2 days to complete this stage. The hatchling measures 4.5 mm to 5 mm (Fig 3.21).

Stage 22 – Tailfin circulation (20 days): Tail fins become transparent. It takes 4 days to complete this stage. The hatchling measures 7 mm (Fig 3.22).

Stage 23 – Operculum covers gill base (24 days): The left and right external gills becomes clearly visible. The length of the gills becomes comparatively shortened. There is appearance of pigment over the whole body. Concentrated pigments formed on the side of the mouth. It takes 4 days to complete this stage. The hatchling measures 8 mm (Fig 3.23a, b, c).

Stage 24 – Operculum closes on right (28 days): Right operculum fold closes and the left external gill shortened. It takes 3 days to complete this stage. The hatchling measures 8.5 mm (Fig 3.24).

Stage 25 – Operculum closes (31 days): After about one month from the time of fertilization, the gills disappeared and there is formation of spiracle which is a single
midlateral opening on the left side. The coiled intestine becomes visible. The parotid
glands are formed behind the eyes. The colour of the tadpole at this stage is beige-
grey. The anterior half of the dorsal side of the tadpole is light beige-grey with a dark
blotch between the eyes starting from just above the nostril. There is a break in the
blotch just posterior to the eye and then it continues again till the anterior part of the
parotid gland. Posterior half of the body is dark brown grey in colour. Caudal muscle
is sandy transparent with small black spots and an ocellus present at the base with a
red halo. The keratodont starts to form at this stage. The development time of the
tadpole at this stage takes about 20 days. During these periods the tadpoles grow into
big size and measures from 11 mm to 30 mm. Keratodont formula is 1:2+2/1+1:3
(Fig 3.25a, b).

Stage 26 – Hindlimb bud starts to develop (51 days): The length of the hindlimb bud
at this stage is less than half its diameter. Limb buds appeared at the junction of the
trunk and tail on either side of the cloacal tail. A large black ocellus with a red halo
is present on the caudal muscle at base of tail followed by two or three smaller ones
of decreasing size. In some individuals, there may be only one ocellus. The parotid
gland is larger than it is in stage 25. In dorsal view, the body is elliptical, widest at
the posterior third; snout semi-circular. In profile, body is depressed; snout rounded.
Eyes are slightly bulging, directed almost laterally and positioned dorsolaterally, not
visible in ventral view. Nares are oval, relatively small-sized, rimmed, with one
anterolateral projection almost dorsally and directed slightly anterolaterally. The
nares are closer to the snout than to pupils. Spiracle is single, sinistral, bulb shaped,
attached to body wall except at its extremity, positioned ventrolaterally, and oriented
more horizontally than posterodorsally. Spiracle opening is oval. Tail musculature
robust, and gradually tapering, almost reaching tail tip. Tail fin moderately high, not extending on to body. A gland is present on the ventral fin, posterior to vent, the infracaudal gland; another at the beginning of dorsal fin, the supracaudal gland, slightly extending on body. Upper fin is slightly higher than lower. Vent tube present. Oral disk large, anteroventral, slightly emarginated, directed ventrally. The limb bud is highly pigmented. It takes 8 days to complete this stage. The tadpole measures 34.1 mm to 64.6 mm. Hindlimb bud measures 0.5 mm. Keratodont formula is 2:4+4/1+1:4 (Fig 3.26).

**Stage 27** – Hindlimb bud is greater than or equal to half its diameter (59 days): There is pigment formation on the limb bud. It takes 5 days to complete this stage. The tadpole measures 39.8 mm to 67.7 mm. Hindlimb bud measures 0.7 mm. Keratodont formula is 2:5+5/1+1:6 (Fig 3.27).

**Stage 28** – Hindlimb bud is greater than or equal to its length (64 days): It takes 8 days to complete this stage. The tadpole measures 48 mm to 71.7 mm. Hindlimb bud measures 0.9 mm. Keratodont formula is 2:4+4/1+1:6 (Fig 3.28).

**Stage 29** – Hindlimb bud is greater than or equal to one and half its diameter (72 days): It takes 7 days to complete this stage. The tadpole measures 48.1 mm to 73.63 mm. Hindlimb bud measures 1.5 mm. Keratodont formula is 2:4+4/1+1:6 (Fig 3.29).

**Stage 30** – Hindlimb bud is equal to twice its diameter (79 days): It takes 9 days to complete this stage. The tadpole measures 49 mm to 75.63 mm. Hindlimb bud measures 1.9 mm. Keratodont formula is 2:4+4/1+1:5 (Fig 3.30).

**Stage 31** - Foot paddle (88 days): The distal end of limb bud curved slightly to form a spatula shape which is referred to as the foot paddle. It takes 10 days to complete
this stage. The tadpole measures 51.5 mm to 76.2 mm Hindlimb bud measures 2 mm. Keratodont formula is 2:5+5/1+1:6 (Fig 3.31).

**Stage 32** – Indentation 4-5 (99 days): The margin of the foot paddle became slightly indented on the dorsal side thus marking the fourth and fifth toe. It takes 9 days to complete this stage. The tadpole measures 54.07 mm to 77.63 mm Hindlimb bud measures 2.5 mm. Keratodont formula is 2:5+5/1+1:6 (Fig 3.32).

**Stage 33** – Indentation 3-4 (108 days): The margin of the foot paddle becomes indented on the ventral side behind the prominence of the fourth toe thus marking the third, fourth and fifth toes. It takes 7 days to complete this stage. The tadpole measures 52.86 mm to 81.63 mm. Hindlimb bud measures 3.2 mm. Keratodont formula is 2:5+5/1+1:6 (Fig 3.33).

**Stage 34** – Indentation 2-3 (115 days): The margin of foot paddle became indented on the ventral side behind the third toe, which marked the prominence of second, third, fourth and fifth toes. It takes 5 days to complete this stage. The tadpole measures 60.2 mm to 91.3 mm. Hindlimb bud measures 3.5 mm. Keratodont formula is 2:5+5/1+1:6 (Fig 3.34).

**Stage 35** – Indentation 1-2 (120 days): The margin of foot paddle became indented behind the second toe demarcating the prominence of first toe. It takes 7 days to complete this stage. The tadpole measures 51.92 mm to 89.82 mm. Hindlimb bud measures 3.85 mm. Keratodont formula is 2:4+4/1+1:6 (Fig 3.35).

**Stage 36** – Toes 3-5 separated (127 days): At this stage, the first and second toes are still joined, while the third, fourth and fifth toes were separated. It takes 6 days to complete this stage. The tadpole measures 71 mm to 90.7 mm. Hindlimb bud measures 4.5 mm. Keratodont formula is 2:4+4/1+1:6 (Fig 3.36).
**Stage 37** – All toes separated (133 days): All five toes were completely separated and there is formation of web between each toes. It takes 12 days to complete this stage. The tadpole measures 61.1 mm to 86.5 mm. Hindlimb measures 6 mm. Keratodont formula is 2:4+4/1+1:5 (Fig 3.37).

**Stage 38** – Metatarsal tubercles (145 days): There is formation of inner and outer metatarsal tubercles. The inner metatarsal tubercle is small, oval and about one fourth of the inner toe. The outer metatarsal tubercle is small and indistinct. It takes 15 days to complete this stage. The tadpole measures 52 mm to 95.72 mm. Hindlimb measures 6.8 mm. Keratodont formula is 2:4+4/1+1:5 (Fig 3.38).

**Stage 39** – Subarticular patches (160 days): As the toes continue to develop, there is formation of the subarticular tubercles which is quite prominent in this species. It takes 15 days to complete this stage. The tadpole measures 56.7 mm to 83.94 mm. Hindlimb measures 8.9 mm. Keratodont formula is 2:4+4/1+1:6 (Fig 3.39).

**Stage 40** – Foot tubercles (175 days): There is complete formation of the foot. Vent tube is present at this stage. It takes 12 days to complete this stage. The tadpole measures 82 mm to 93 mm. Hindlimb measures 13.7 mm. Keratodont formula is 2:4+4/1+1:6 (Fig 3.40).

**Stage 41** – Forelimb visible (187 days): It takes 7 days to complete this stage. The forelimbs have not emerged as yet but they are fully formed and are visible through the skin. The vent tube is absent at this stage. The tadpole measures 52.9 mm to 91.64 mm. Hind limb measures 34 mm. Keratodont formula is 2:5+5/1+1:6 (Fig 3.41).

**Stage 42** – Forelimb emerged (194 days): It takes 2 days to complete this stage. The left forelimb emerged first and after some hours which may continue to a day or two,
the right forelimb will emerge. This may be due to the fact that the spiracle opening is situated on the left side. The tadpole measures 69.4 mm to 92.2 mm. Hind limb measures 41 mm. The keratodonts starts to degenerate (Fig 3.42 a, b).

**Stage 43** – Tail atrophies (196 days): It takes 7 days to complete this stage. The forelimbs and the hindlimbs are fully developed and the tail continues to regress. At this stage, the angle of jaw is between the nostril and the eye. Total body length is 35 mm to 83.5 mm. Hind limb measures 45 mm. Mouth developed (Fig 3.43).

**Stage 44** – Tail greatly reduced (203 days): It takes 5 days to complete this stage. The mouth continues to widen and at this stage the angle of the jaw is seen beneath the eye and the tail is greatly reduced at this stage. The tadpole measures 48.3 mm to 93.8. Hind limb measures 65.36 mm (Fig 3.44).

**Stage 45** – Mouth posterior to eye (208 days): It takes 7 days to complete this stage. The angle of jaw is seen posterior to the eye and tail stub is still present at this stage. The tadpole measures 37 mm to 61 mm. Hind limb measures 72.96 mm (Fig 3.45).

**Stage 46** – Metamorphosis completed (215 days): After 7 months from the time of fertilization, the tadpole finally metamorphosed to a froglet. The froglet measures 23 mm to 39.1 mm at the time of metamorphosis. Hind limb measures 68 mm (Fig 3.46).

The complete developmental time of *Rana alticola* takes 215 days which is approximately seven months. Breeding activities in this species starts late June and continue till early October. The eggs are deposited in multiple clutches attached to vegetations which are present around the breeding site. The egg at the time of laying measures about 1.2 mm to 1.5 mm in diameter. The egg starts to change in shape from stage 11 onwards. From stage 13 (i.e. Neural plate) onwards, it is referred to as
embryo since it starts to elongate. Hatching takes place at stage 19 (i.e. heartbeat stage) after about 13 days and 20 hours, it is now referred to as hatchling. The operculum closes on day 28 when the hatchling reaches stage 24. From stage 25 onwards, it is referred to as tadpole. The hindlimb buds starts to grow from stage 26 after 51 days from the time of fertilization. The tadpole continues to grow and there is development of the hind limbs and finally at stage 42, the forelimbs emerged after 194 days and finally complete metamorphosis takes place after 215 days from the time of fertilization (Table 3.1).

The ecological factors from the three study sites during the study periods from 2005 to 2007 were recorded. The development and metamorphosis of the tadpole was observed from September 2005 to April 2006 and from September 2006 to April 2007. At study site I, from September 2005 to April 2006 the rainfall ranged from 6 mm to 372 mm. No rainfall was observed in December 2005 and January and February in 2006. From September 2006 to April 2007, the rainfall ranged from 11 mm to 331 mm. No rainfall was observed in December 2006 and January 2007. At study site II, from September 2005 to April 2006 the rainfall ranged from 1.2 mm to 383.8 mm. No rainfall was observed in November and December 2005 and January and February in 2006. From September 2006 to April 2007, the rainfall ranged from 8.3 mm to 360.8 mm. No rainfall was observed in December 2006 and January 2007. At study site III, from September 2005 to April 2006 the rainfall ranged from 37.3 mm to 308.3 mm. No rainfall was observed in December 2005 and January, February and March in 2006. From September 2006 to April 2007, the rainfall ranged from 39 mm to 390.7 mm. No rainfall was observed in November and December 2006 and January 2007 (Table 2.1, Fig 3.48, 3.49, 3.50).
The relative humidity at study site I from September 2005 to April 2006 ranged from 26% to 95% and from September 2006 to April 2007, ranged from 31% to 94%. The relative humidity at study site II, from September 2005 to April 2006, ranged from 42% to 94% and from September 2006 to April 2007, ranged from 50% to 98%. The relative humidity at study site III, from September 2005 to April 2006, ranged from 45% to 88% and from September 2006 to April 2007, ranged from 50% to 95% (Table 2.2; Fig 3.51, 3.52, 3.53).

The air temperature at study site I from September 2005 to April 2006 ranged from 12°C to 35°C and from September 2006 to April 2007, ranged from 13°C to 31°C. The air temperature at study site II from September 2005 to April 2006 ranged from 14°C to 35°C and from September 2006 to April 2007, ranged from 14°C to 33°C. The air temperature at study site III from September 2005 to April 2006 ranged from 17°C to 31°C and from September 2006 to April 2007, ranged from 18°C to 31°C (Table 2.3; Fig 3.54, 3.55, 3.56).

The water temperature at study site I from September 2005 to April 2006 ranged from 10°C to 23°C and from September 2006 to April 2007, ranged from 10°C to 23°C. The water temperature at study site II from September 2005 to April 2006 ranged from 11°C to 25°C and from September 2006 to April 2007, ranged from 12°C to 22°C. The water temperature at study site III from September 2005 to April 2006 ranged from 11°C to 23°C and from September 2006 to April 2007, ranged from 11°C to 21°C (Table 2.4; Fig 3.57, 3.58, 3.59).

The pH at study site I from September 2005 to April 2006 ranged from 6.6 to 7.6 and from September 2006 to April 2007, ranged from 6.6 to 7.3. The pH at study site II from September 2005 to April 2006 ranged from 6.4 to 7 and from September
2006 to April 2007, the pH ranged from 6.5 to 7. The pH at study site III from September 2005 to April 2006 ranged from 6.7 to 7 and from September 2006 to April 2007, ranged from 6.6 to 7.2 (Table 2.5; Fig 3.60, 3.61, 3.62).

It was found that during the development period i.e from September 2005 to April 2006 and September 2006 to April 2007, rainfall start to decrease in all the three study sites and the level of the water in the study sites have subsided and the current of the water is comparatively slow (Fig 2.11b, 2.12b and 2.13b)
DISCUSSION

Literature surveys on *Rana alticola* revealed that there is no work on its development and metamorphosis except for some works on the tadpoles by Sahu and Khare (1980) who published field key of *Rana alticola* tadpoles and also by Grojean *et al.*, (2003) who gave the morphology and buccopharyngeal anatomy of the tadpole of *Rana (Nasirana) alticola*. The present study revealed that, in Mizoram, the development and metamorphosis of *Rana alticola* takes 215 days which is approximately seven months. Species spawning in permanent water tend to have prolonged larval periods and metamorphose at sizes larger than temporary-pond spawners (Patterson and McLachlan 1989), similarly *Rana alticola* breed in both lentic and lotic environment. The developmental time of *Rana alticola* is comparatively long as compared to other Ranids like *Rana erythraea* which is estimated to take between 50 and 82 days total larval period up to metamorphosis (Leong and Chou, 1999). *Rana breviceps* takes 45 days to complete its life history (Mohanti Hejmadi *et al.*, 1979a). In Orissa *Rana (Euphlyctis) cyanophlyctis* takes approximately 46 days at 32 °C – 41 °C to complete metamorphosis (Mohanty-Hejmadi and Dutta, 1978). *Rana longicrus*, which is a winter breeder from Taiwan takes 50 – 60 days at water temperature 19 °C – 20 °C, (Yuan, 1950: Kam *et al.*, 1995). Dutta and Mohanty-Hejmadi (1976) reported that the Indian bull frog *Rana (Hoplobatrachus) tigerina* takes 33 days to complete its life history in the laboratory condition at temperature 28°C - 36 °C. It may be suggested that, the longer time taken for *Rana alticola* for development could be due to the time of development that coincides with cold climate where the water temperature ranges from 10°C - 25°C (i.e from September 2005 to April 2006 and September 2006 to April 2007).
While many studies on the effects of temperature on tadpole biology have been performed on temperate species such as *Rana pipsiens* (Casterlin and Reynolds, 1979) or *R. catesbeiana* (Lillywhite, 1970; Menke and Claussen, 1982), less is known about the effects of temperature on the tadpoles of tropical frogs.

The eggs of *Rana alticola* at the time of laying measures about 1.2 mm to 1.5 mm in diameter which is more or less similar to other Ranid eggs like *Rana cancrivora* eggs with average of 1.2-1.3 mm (Alcala 1962). *Rana breviceps* egg measures 1-1.2 mm (Mohanti Hejmadi et al., 1979a) and the Indian bull frog *Rana (Hoplobatrachus) tigerina* eggs ranges from 1.1-1.8 mm (Dutta and Mohanty-Hejmadi, 1976). However, the eggs of *Rana plicatella* are relatively large at 1.8-2.1mm (Leong and Chou, 1999).

Hatching in *Rana alticola* was observed after 13 days at stage 19 (i.e heartbeat stage) which is comparatively late as compared to the other ranids of India like the Indian bull frog *Rana (Hoplobatrachus) tigerina* which hatched at 24 hrs (Dutta and Mohanty-Hejmadi, 1976) and also the Indian burrowing frog *Rana breviceps* where hatching takes place in about 44 hrs (Mohanty-Hejmadi et al., 1979a).

During the process of embryonic development and metamorphosis, it was observed that the pH value ranged between 6.4 – 7.6 at the three study sites (i.e, from September 2005 to April 2006 and September 2006 to April 2007) which were located in the undisturbed areas away from anthropogenic activities, and also in the laboratory. The pH range (6.4 – 7.6) appears to be optimal for the normal embryonic development and metamorphosis of *Rana alticola*. It appears that acidification of habitat has a major impact on amphibians and the structure of their populations.
where field studies on amphibian abundance and species diversity have shown a clear correlation between the acidification of breeding ponds and the decline of amphibian populations (Beebee, 1987). Although there was a negative effect on these life-history parameters in some studies (Rowe et al., 1992; Beebee, 1986), others failed to find any effects (Ling et al., 1986; Kiesecker, 1996). Rowe and Freda (2000) reported that at slightly higher levels of pH, embryonic development proceeds, yet events occurring later in development may prevent hatching, therefore trapping and often killing the embryo. Increasing habitat acidification presumably exerts a strong selection pressure on individuals of the respective populations. However, very little is known about the potential of amphibians to adapt to low pH conditions (Andren et al., 1989). Glos et al., (2003) reported that low pH treatment on the population of Rana temporaria caused a prolongation in embryogenesis and an increased embryonic mortality, a higher proportion of deformed hatchlings and an increased larval time. In the larval stage, the pH tolerance of R. temporaria was greater than in the embryonic stage as shown in other anuran species (Beebee, 1986). Low environmental pH can also cause death of amphibian embryos by a selective effect that leads to constriction of the extra-embryonic membranes and severe curling of the embryo (Dunson and Connell, 1982). Although some populations of certain species show a greater tolerance to low pH (Clark and LaZerte, 1987; Gosner and Black, 1957), this is not a general phenomenon (Clark, 1986). Additional studies on adaptations to low pH and other parameters of habitat acidity are of great importance, for both estimating chances of survival of amphibian populations in acidified habitats and for applying appropriate means for conservation.
Tadpoles from different localities referred to *Rana alticola* have been
described by previous authors like Annandale (1912) where he reported a size of at
least 57 mm without specifying the developmental stage of the tadpole, Smith
(1924a) reported a size of 96 mm for the peninsular Thailand population, Sahu &
Khare (1980) also reported a maximum size of 98 mm at stage 38 for the population
of northeastern hills region, India and Grosjean *et al.*, (2003) reported the size of 93
mm from Phang Nga Province, Thailand. The measurements of total length given by
these authors fit well with the present observations: a maximum of 95.72 mm at
stage 38 collected from Mizoram, North East India. The tadpoles of *Rana alticola*
are larger in size in comparison to other Ranid tadpoles which is in agreement with
laboratory studies which show a pattern where tadpoles growing at low temperatures
develop more slowly but eventually metamorphose at a larger size (Etkin, 1964;
Smith-Gill and Berven, 1979; Hayes *et al.*, 1993). *Rana alticola* tadpoles are found
both in the ponds as well as stream and rivers and the tadpoles which are found in
streams and river usually occupy quiet areas where the current is slow and are
therefore conspicuous because of their large size and black coloration.

As observed in the tadpoles of *Rana alticola*, tadpoles of *Nasirana* possess
several conspicuous features, including the tail ocellus, which is unique among ranid
tadpoles. As far as known, no other ranid species have a similar feature, and that is
sufficient to differentiate this tadpole species from all other ranid species. *Rana
alticola* also possesses other conspicuous features such as paratoid glands and other
glands which are shared by the tadpoles of subgenera *Clinotarsus* Mivart, 1869,
*Glandirana* Fei, Ye and Huang, 1990 and *Sanguirana* Dubois, 1992. The caudal
ocelli and the glands present in *Rana alticola* could be interpreted as means of
defense as reported by some authors where glands secreting noxious substances are known to be efficient against predators (Liem, 1961) whereas the largest red ocellus is thought to mimic the eye of a larger animal (Altig and Channing, 1993) and could misdirect predator attacks. Usefulness of the numerous keratodonts rows present in Rana alticola is more difficult to explain since these tadpoles inhabit slow water areas. However, this occurs frequently in stream living tadpoles (Altig and Johnston, 1989).

Differences in coloration and number of ocelli have been noted by most of the previous authors which was also observed in the population of Mizoram. Although the centres of ocelli are invariably black, the coloration of the outer ring varies from yellow (Annandale, 1912) to red (Grosjean et al., 2003) to orange (Smith, 1924a), these authors did not indicate if this coloration was from living or fixed tadpoles. It is thus not possible to establish if these differences in coloration are due to interpopulational variation or, more likely, to a fading in preservative. Boulenger (1882), Smith (1924a), Bourret (1942) and Sahu & Khare (1980) reported one ocellus on each side of the tadpole. However, Annandale (1912) and Grosjean et al., (2003) reported the presence of several ocelli which is also observed in some of the tadpoles of Rana alticola collected from Mizoram.

In the present investigation, it was found that Rana alticola developed and metamorphosed successfully in the water temperature ranging from of 10°C - 25°C and water pH ranging from 6.4 - 7.5 from September 2005 to April 2006 and September 2006 to April 2007. This suggests that relatively low temperature do not have adverse effect on the development and metamorphosis of this species.
Table 3.1: Developmental stages (Gosner 1960) of *Rana alticola* with age and size

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>Stage</th>
<th>Age</th>
<th>Size (mm)</th>
<th>Hind limb (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Fertilization</td>
<td>0 hr</td>
<td>1.2 - 1.5</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>Gray Crescent</td>
<td>30 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>2-cell</td>
<td>5 hrs</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>4-cell</td>
<td>6 hrs 30 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>8-cell</td>
<td>8 hrs 11 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>16-cell</td>
<td>10 hrs 25 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>32-cell</td>
<td>12 hrs 33 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>Mid Cleavage</td>
<td>14 hrs 50 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>Late Cleavage</td>
<td>16 hrs 50 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>Dorsal Lip</td>
<td>44 hrs</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>Mid gastrula</td>
<td>48 hrs 25 mins</td>
<td>1.3 - 1.6</td>
<td></td>
</tr>
<tr>
<td>12.</td>
<td>Late Gastrula</td>
<td>58 hrs 35 mins</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>13.</td>
<td>Neural Plate</td>
<td>4 days</td>
<td>1.45 - 1.75</td>
<td></td>
</tr>
<tr>
<td>14.</td>
<td>Neural Fold</td>
<td>5 days</td>
<td>1.5 - 1.8</td>
<td></td>
</tr>
<tr>
<td>15.</td>
<td>Rotation</td>
<td>6 days 10 hrs</td>
<td>1.5 - 1.8</td>
<td></td>
</tr>
<tr>
<td>16.</td>
<td>Neural Tube</td>
<td>7 days 20 hrs</td>
<td>1.7 - 2</td>
<td></td>
</tr>
<tr>
<td>17.</td>
<td>Tail Bud</td>
<td>9 days</td>
<td>1.8 - 2.1</td>
<td></td>
</tr>
<tr>
<td>18.</td>
<td>Muscular Response</td>
<td>12 days</td>
<td>2.3 - 2.5</td>
<td></td>
</tr>
<tr>
<td>19.</td>
<td>Heart Beat</td>
<td>13 days 20 hrs</td>
<td>3.5 - 3.6</td>
<td></td>
</tr>
<tr>
<td>20.</td>
<td>Tail Elongation</td>
<td>16 days 15 hrs</td>
<td>3.8 - 4</td>
<td></td>
</tr>
<tr>
<td>21.</td>
<td>Cornea Transparent</td>
<td>18 days 10 hrs</td>
<td>4.5 - 5</td>
<td></td>
</tr>
<tr>
<td>22.</td>
<td>Tail Fin Circulation</td>
<td>20 days</td>
<td>6.5 - 7</td>
<td></td>
</tr>
<tr>
<td>23.</td>
<td>Operculum covers gill bases</td>
<td>24 days</td>
<td>7.5 - 8</td>
<td></td>
</tr>
<tr>
<td>24.</td>
<td>Operculum closes on right</td>
<td>28 days</td>
<td>8 - 8.5</td>
<td></td>
</tr>
<tr>
<td>25.</td>
<td>Spiracles Forms</td>
<td>31 days</td>
<td>11 - 30</td>
<td></td>
</tr>
<tr>
<td>26.</td>
<td>L &lt; (\frac{1}{2}D)</td>
<td>51 days</td>
<td>34.1 - 64.6</td>
<td>0.5</td>
</tr>
<tr>
<td>27.</td>
<td>L = (\frac{1}{2}D)</td>
<td>59 days</td>
<td>39.8 - 67.7</td>
<td>0.7</td>
</tr>
<tr>
<td>28.</td>
<td>L = D</td>
<td>64 days</td>
<td>48 - 71.7</td>
<td>0.9</td>
</tr>
<tr>
<td>29.</td>
<td>L = (1\frac{1}{2}D)</td>
<td>72 days</td>
<td>48.1 - 73.63</td>
<td>1.5</td>
</tr>
<tr>
<td>30.</td>
<td>L = 2D</td>
<td>79 days</td>
<td>49 - 75.63</td>
<td>1.9</td>
</tr>
<tr>
<td>31.</td>
<td>Foot Paddle</td>
<td>88 days</td>
<td>51.5 - 76.2</td>
<td>2</td>
</tr>
<tr>
<td>32.</td>
<td>Indentation 4-5</td>
<td>99 days</td>
<td>54.07 - 77.63</td>
<td>2.5</td>
</tr>
<tr>
<td>33.</td>
<td>Indentation 3-4</td>
<td>108 days</td>
<td>52.86 - 81.63</td>
<td>3.2</td>
</tr>
<tr>
<td>34.</td>
<td>Indentation 2-3</td>
<td>115 days</td>
<td>60.2 - 91.3</td>
<td>3.5</td>
</tr>
<tr>
<td>35.</td>
<td>Indentation 1-2</td>
<td>120 days</td>
<td>51.92 - 89.82</td>
<td>3.85</td>
</tr>
<tr>
<td>36.</td>
<td>Toes 3-5 Separated</td>
<td>127 days</td>
<td>71 - 90.7</td>
<td>4.5</td>
</tr>
<tr>
<td>37.</td>
<td>All Toes Separated</td>
<td>133 days</td>
<td>61.1 - 86.5</td>
<td>6</td>
</tr>
<tr>
<td>38.</td>
<td>Metatarsal tubercles</td>
<td>145 days</td>
<td>52 - 95.72</td>
<td>6.8</td>
</tr>
<tr>
<td>39.</td>
<td>Sub-articular patches</td>
<td>160 days</td>
<td>56.7 - 83.94</td>
<td>8.9</td>
</tr>
<tr>
<td>40.</td>
<td>Vent tube present</td>
<td>175 days</td>
<td>82 - 93</td>
<td>13.7</td>
</tr>
<tr>
<td>41.</td>
<td>Fore Limbs Visible</td>
<td>187 days</td>
<td>52.9 - 91.64</td>
<td>34</td>
</tr>
<tr>
<td>42.</td>
<td>Fore Limbs emerge</td>
<td>194 days</td>
<td>69.4 - 92.2</td>
<td>41</td>
</tr>
<tr>
<td>43.</td>
<td>Tail Atrophies</td>
<td>196 days</td>
<td>35 - 83.5</td>
<td>45</td>
</tr>
<tr>
<td>44.</td>
<td>Tail Greatly Reduced</td>
<td>203 days</td>
<td>48.3 - 93.8</td>
<td>65.36</td>
</tr>
<tr>
<td>45.</td>
<td>Tail Stub</td>
<td>208 days</td>
<td>37 - 61</td>
<td>72.96</td>
</tr>
<tr>
<td>46.</td>
<td>Metamorphosis Complete</td>
<td>215 days</td>
<td>23 - 39.1</td>
<td>68</td>
</tr>
</tbody>
</table>
Fig 3.1: Stage 1 - Fertilization
Fig 3.2: Stage 2 - Gray crescent
Fig 3.3: Stage 3 - Two cell stage

Fig 3.4: Stage 4 - Four cell stage
Fig 3.5: Stage 5 - Eight cell stage
Fig 3.6: Stage 6 - Sixteen cell stage
Fig 3.7: Stage 7 - Thirty two cell stage
Fig 3.8: Stage 8 - Mid cleavage
Fig 3.9: Stage 9 - Late cleavage
Fig 3.10: Stage 10 - Dorsal lip
Fig 3.11: Stage 11 - Mid Gastrula
Fig 3.12: Stage 12 - Late Gastrula
Fig 3.13 : Stage 13 - Neural plate
Fig 3.14 : Stage 14 - Neural folds
Fig 3.15 : Stage 15 - Rotation
Fig 3.16 : Stage 16 - Neural tube
Fig 3.17 : Stage 17 - Tail bud
Fig 3.18 : Stage 18 - Muscular response
Fig 3.19: Stage 19
Heart beat

Fig 3.20: Stage 20
Gill circulation

Fig 3.21: Stage 21
Cornea transparent

Fig 3.22: Stage 22
Tailfin circulation
Fig 3.23a : Stage 23 - Operculum covers gill bases [Dorsal view]
Fig 3.23b : Stage 23 - Ventral view
Fig 3.23c : Stage 23 - Lateral view

Fig 3.24 : Stage 24 - Operculum closes on right (Dorsal view)
Fig 3.25a: Stage 25 - Operculum fold closes on left; spiracle form (Ventral view) [Coiled intestine becomes visible; Mouthparts obvious]

Fig 3.25b: Stage 25 - Dorsal view
Fig 3.26 : Stage 26 - L < ½ D
Fig 3.27 : Stage 27 - L ≥ ½ D
Fig 3.28 : Stage 28 - L ≥ D
Fig 3.29 : Stage 29 - L ≥ 1½ D
Fig 3.30 : Stage 30 - L = 2 D
Fig 3.31 : Stage 31 - Foot paddle
Fig 3.32: Stage 32 - Indentation 4-5
Fig 3.33: Stage 33 - Indentation 3-4
Fig 3.34: Stage 34 - Indentation 2-3
Fig 3.35: Stage 35 - Indentation 1-2
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Fig 3.37: Stage 37 - All toes separated
Fig 3.38: Stage 38 - Metatarsal tubercle
Fig 3.39: Stage 39 - Subarticular patches
Fig 3.40: Stage 40 - Vent tube present
Fig 3.41: Stage 41 - Fore limbs visible
Fig 3.42a: Stage 42 - Left forelimb emerge first
Fig 3.42b: Stage 42 - Both forelimbs emerged
Fig 3.43: Stage 43 - Tail atrophies

Fig 3.44: Stage 44 - Tail greatly reduced

Fig 3.45: Stage 45 - Tail stub

Fig 3.46: Stage 46
Metamorphosis complete
Fig 3.47 (a & b): Enclosures to study the development of *Rana alticola* in the field.
Fig 3.48: Monthly variation of rainfall at study site I

Fig 3.49: Monthly variation of rainfall at study site II

Fig 3.50: Monthly variation of rainfall at study site III
Fig 3.51: Monthly variation of relative humidity at study site I

Fig 3.52: Monthly variation of relative humidity at study site II

Fig 3.53: Monthly variation of relative humidity at study site III
Fig 3.54: Monthly variation of Air temperature at study site I

Fig 3.55: Monthly variation of Air temperature at study site II

Fig 3.56: Monthly variation of Air temperature at study site III
Fig 3.57: Monthly variation of Water temperature at study site I

Fig 3.58: Monthly variation of Water temperature at study site II

Fig 3.59: Monthly variation of Water temperature at study site III
Fig 3.60: Monthly variation of pH at study site I

Fig 3.61: Monthly variation of pH at study site II

Fig 3.62: Monthly variation of pH at study site III
Fig 4.1: Oral structure of stage 25 of *Rana alticola* LTRF 1:2+2/1+1:2

Fig 4.2: Oral structure of stage 26 of *Rana alticola* showing marginal teeth LTRF 2:5+5/1+1:7