

STUDIES ON THE ECOLOGY AND BIOLOGY OF
Rana alticola (BOULENGER)

SAIPARI SAILO

DEPARTMENT OF ZOOLOGY
NORTH EASTERN HILL UNIVERSITY
SHILLONG - 793022

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Rana alticola (BOULENGER)

BY

SAIPARI SAILO

DEPARTMENT OF ZOOLOGY

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THE NORTH EASTERN HILL UNIVERSITY

SHILLONG – 793022

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DECLARATION

I, Miss Saipari Sailo, hereby declare that the subject matter of this thesis is the record of the work done by me, that the contents of this thesis did not form the basis of the award of any previous degree to me or to the best of my knowledge to anybody else, and that the thesis has not been submitted by me for any research degree in any other University /Institute.

This is being submitted to the North-Eastern Hill University for the degree of Doctor of Philosophy in Zoology.

[Prof. (Mrs.) R. N. K. Hooroo]

Supervisor and Head

(Miss Saipari Sailo)

Candidate

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GENERAL INTRODUCTION

Amphibians are a class of vertebrates that generally make a change in habitat at some point of their lives (Just *et al.*, 1981). Such a change normally involves the transformation from an aquatic larva to a terrestrial adult. The word “Amphibia” literally means double life and was first used by Linnaeus and refers to the ability of these animals to live in water or on land at different times. Amphibians are intermediate in some ways between the fully aquatic fishes and the terrestrial amniotes. However, they are not simply transitional in their morphology, life history, ecology, and behaviour. In the successful attainment of independence from water and colonization of land, amphibians have undergone a remarkable adaptive radiation, and the living groups exhibit a great diversity of modes of life history than any other group of vertebrates.

Amphibians can be grouped into three orders. These are Anura, Urodela and Caecilia. Frogs and toads fall under the order anura and they are the largest group of amphibians and have the broadest geographical distribution, about 85% of amphibians. Salamanders fall under the order Caudata and differ from other amphibians in having a tail even after metamorphosis from the larval stage. Also known as tailed amphibian and constitute 10% of amphibians. Caecilians are the only living amphibians that are completely legless. They are also known as limbless amphibians and they constitute 5% of amphibians. The term Urodela and Apoda are sometimes used instead of Caudata and gymnophiona for salamanders and caecilians respectively, whereas in some literature Salientia is used interchangeably with Anura. Amphibians currently include 6,662 recognized species with representatives found in virtually all terrestrial and freshwater habitats, in all but the coldest and

driest regions or the most remote oceanic islands. Since 1985 the total number of recognized species has increased by over 60% (Frost 2010). This growth reflects the increasing ease of collecting in remote locations and a significant growth of active scientific communities in a few megadiverse countries. Unfortunately, the rapid increase in knowledge of amphibian species diversity is coincident with a massive and global decline in amphibian populations (Alford and Richards, 1999; Houlahan *et al.*, 2000; Young *et al.*, 2001; Stuart *et al.*, 2004) due to a diversity of factors, including habitat loss and fragmentation (Green, 2005; Halliday, 2005) but also possibly due to global environmental changes (Donnelly and Crump, 1998; Blaustein and Kiesecker, 2002; Heyer, 2003; Licht, 2003) and such proximate causes as emerging infectious diseases (Collins and Storfer, 2003).

The diversity of reproductive modes in amphibians is much greater than that observed in other groups of vertebrates, especially the amnoites. In each of the three living orders of Amphibia there are trends towards terrestriality. The variety of these trends is especially noteworthy in anurans. These reproductive adaptations have been viewed as pioneering evolutionary experiments in the conquest of terrestrial environments by vertebrates (Goin, 1960). In most anurans, fertilization occurs externally at the time of oviposition and the pair is in amplexus and there is some evidence for various kinds of tactile signals between the sexes.

The vocalization of frogs have provided a means for studying acoustic communication and, together with other aspects of courtship and mating, have presented the evolutionary biologists with a wealth of material for studies on sexual selection. Among vertebrates, vocalization is highly developed in anurans, birds, bats, primates, cetaceans, and dolphins. In the anurans and birds the primary purpose

of vocalization is advertisement. Sound production by animals is primarily a method of advertising the presence of one individual to others of the same species.

The life histories of amphibians are highly diversified. Most species of frogs have external fertilization, whereas internal fertilization occurs in the majority of salamanders and presumably in all caecilians. All amphibian eggs must develop in moist situation, for although they have numerous protective mucoid capsules, these capsules are highly permeable. The eggs lack a shell and the embryonic membranes of higher vertebrates. Their shell-less eggs have been studied extensively by developmental biologists, and much of the basic knowledge of vertebrate embryology is based on amphibians. The metamorphosis from aquatic larvae to terrestrial adults has been the subject of intensive studies.

Amphibians are generally considered to be feeding opportunists with their diets reflecting the availability of food of appropriate size. The limited information on amphibian diets indicates that all adult amphibians are carnivores; most feed principally on insects, although many species eat a wide variety of invertebrates.

Anurans are the largest group of amphibians. Approximately 5,891 species are in this group (Frost 2010), and they reside on all of the major continents except Antarctica and on many oceanic islands (Pough *et al.*, 2004; Zug *et al.*, 2001). The frog family Ranidae (sensu Frost *et al.*, 2006, equivalent to Raninae sensu Bossuyt *et al.*, 2006) contains over 300 species and occurs in most temperate and tropical parts of the world (Frost, 2007). *Rana* is the largest genus of ranids. The family exhibits tremendous ecological, morphological, and developmental diversity across its wide geographic range (Stuart 2008).

Rana alticola, (Fig 1.10 and 1.11 a, b) also known as Plain Oriental Stream frog falls under the order anura and belong to the family Ranidae. Ranidae are also known as true frogs, typically, true frogs are smooth, moist-skinned frogs, with large, powerful legs and extensively webbed feet. Many of the true frogs are aquatic or live close to water. Most species lay their eggs in the water and go through a tadpole stage. However, as with most families of frogs, there is large variation of habitat within the family. The adults of *Rana alticola* are extremely rare and nocturnal but their tadpoles are abundantly available (Chanda, 1994). Sahu and Khare (1980) published field key of *Rana alticola* tadpoles and also on the food and feeding habits of *Rana alticola* during different stages of metamorphosis (Sahu and Khare 1988) and Grojean *et al.*, (2003) gave the morphology and buccopharyngeal anatomy of the tadpole of *Rana (Nasirana) alticola*. It may be mentioned that Pawar and Birand (2001) reported the presence of *Rana alticola* from Ngengpui Wildlife Sanctuary situated in the Saiha District of Mizoram, and Sen (2004) also reported its occurrence in Mizoram without mentioning the specific area of collection from the state. Other than these works, little is known about the ecology and biology of the species. Studies regarding its distribution, habit and habitat, breeding behaviour, development and food and feeding behaviour of *Rana alticola* in Mizoram or other parts of adjoining areas in North Eastern India and from other parts of the globe are not available. Therefore, it is envisaged to take up a study on these aspects to know more about the distribution, habit and habitat, breeding behaviour, development and food and feeding behaviour of *Rana alticola* in Mizoram.

OBJECTIVES: The proposed study on *Rana alticola* include the following objectives:-

1. Study of the Distribution, habit and habitat, food and feeding behaviour and abiotic factors such as temperature, rainfall, pH and humidity in Mizoram.
2. Study of breeding behavior, spawning and nesting.
3. Study of the developmental stages with reference to morphometric changes of the tadpoles.

NOMENCLATURE OF THE SPECIES

The original name of the species was *Rana alticola* described by Boulenger in 1882. Hence, the name *Rana alticola* was used in the thesis title. However, the recent taxonomic resolution adopted by Frost *et al.*, (2006) maintained the species under the genus *Nasirana*. Stuart (2008) mentioned that the monotypic genera *Clinotarsus* and *Nasirana* are strongly supported sister species that share several unique larval characters (Hiragond *et al.*, 2001; Grosjean *et al.*, 2003), and continuing to recognize both genera is unnecessary. *Nasirana* Dubois, 1992 should therefore be treated as a junior synonym of *Clinotarsus* Mivart, 1869. Hence the present nomenclature of the species is *Clinotarsus alticola* (Boulenger)

The presentation of these studies in the thesis has been divided into the following chapters.

Chapter I: Distribution of *Rana alticola* in Mizoram.

Chapter II: Habit, habitat and breeding behaviour of *Rana alticola*.

Chapter III: Development of *Rana alticola*

Chapter IV: Oral structure of the tadpoles and the food and feeding habits of both the tadpoles and adults.

Conclusion

References

Chapter I

Distribution of *Rana alticola* in Mizoram

INTRODUCTION

Rana alticola was originally recorded from Khasi Hills, Meghalaya by (Boulenger, 1882). Pillai and Chanda (1979) recorded the species for the second time from Meghalaya following its first record by Boulenger in 1882. In North East India, *Rana alticola* has been reported from Meghalaya, Assam, Nagaland, Mizoram and Tripura. Besides these, the distribution of *Rana alticola* in India includes West Bengal, Orissa and Andaman. Outside India, it has been reported from Sri Lanka, Nepal, China, Japan, Indonesia, Bangladesh, Myanmar, Thailand and Malaysia. Pawar and Birand (2001) and Sen (2004) reported the occurrence of *Rana alticola* from Mizoram. In the present investigation, the state of Mizoram, in North East India has been chosen as the study site and a thorough survey was conducted to know the distribution of *Rana alticola* in different parts of Mizoram.

Mizoram, situated in the southernmost state in North-east India, is one of the mega Biodiversity hotspots of the world and lies between 21°56'N – 24°31'N and 92°16'E – 93°26' E. Mizoram is bounded by the state of Manipur and the Cachar District of Assam to its north, the Chin Hills of Myanmar to its east, by the Arakan Hill ranges of Myanmar to the South and by Tripura State and the Chittagong Hills of Bangladesh on the West. The state is divided into eight districts – Aizawl, Mamit, Serchhip, Champhai, Kolasib, Lunglei, Saiha and Lawngtlai (Fig 1.1). The geomorphology of Mizoram is dominated by a series of parallel hill ranges, generally running from north to south, increasing in elevation from west to east. The numerous rivers, governed by the hill ranges flow either from north to south, or *vice versa*, often following a tortuous course. This creates a complex drainage pattern with several parallel rivers flowing in opposite directions. The hill ranges can be

classified into the ridge and valley province (Altitudinal range of 40 – 1550 m), occupying most of the state and the mountainous terrain province (Altitudinal range of 400-2157m), restricted to an eastern longitudinal strip adjoining Myanmar (Pachua, 1994; Singh, 1996).

The family Ranidae is one of the most species-rich amphibian families. It is distributed throughout the world, except southern South America and most of Australia (Frost, 1985, Duellman, 1993). With more than 1000 extant species, Ranidae *sensu lato* (i.e., *sensu* Dubois, 2005) is one of the largest extant families of anurans. This cosmopolitan group of frogs evolved an enormous diversity in all kinds of habitats, such as forests, savannas, grasslands, and deserts. The characters associated with this ecological, morphological, and developmental diversity have been utilized extensively by systematists for generating hypotheses on ranid relationships (Clarke, 1981; Dubois, 1992; Blommers- Schlösser, 1993; Inger, 1996). In recent years, the integration of molecular phylogenetic studies (Bossuyt and Milinkovitch, 2000; Emerson *et al.*, 2000; Roelants *et al.*, 2004) led to revised classifications, recognizing several subfamilies with a wide distribution (e.g., Dicroglossinae, Raninae) (Dubois, 2003; Frost, 2004). The subcosmopolitan frog family Ranidae is ecologically an extremely diverse amphibian group, represented by approximately 500 species in the Oriental realm (Inger, 1999; Meegaskumbura *et al.*, 2002). Thus the study on the distribution of *Rana alticola*, a member of the the family Ranidae in Mizoram, North East India is relevant.

REVIEW OF LITERATURE

Review of literature shows that a number of reports, monograph and books have been published on the taxonomy and distribution of amphibians. The fauna of British India including Ceylon and Burma by Boulenger (1890), the amphibian fauna of Thailand by Taylor (1962), A monograph of the South Asian, Papuan, Melanesian and Australian frogs of the genus *Rana* by Boulenger (1920), Living Amphibians of the World by Cochran (1967), Zoogeography of Indian amphibians: Distribution, diversity and spatial relationship (Tiwari, 1991), Vernacular names of some Southeast Asian amphibians and reptiles by Das (1993), Checklist and bibliography on amphibians of India and Sri Lanka Dutta (1997). Patterns of Distribution of amphibians: A global perspective by Duellman (1999), Amphibians of Peninsular India by Daniels (2005), The Amphibian Tree of Life by Frost *et al.*, (2006).

The Indian sub-continent is an admixture of Gondwana relics, Oriental, Ethiopian and Palearctic elements; it is clearly reflected in the diversity of amphibian fauna in the region. The amphibian fauna of India finds a special place in the context of Global Amphibian Conservation because of this unique feature. The first ever report on the amphibians of India was published in the form of a report 'The reptiles of British India' by Gunther (1864) in which, 37 anurans (toads and frogs) and 2 caecilians (limbless amphibians) are mentioned. This was followed by the seminal work on the group by Boulenger (1890), which till date stands as the most authoritative account of the amphibian taxonomy of British India. His work included 124 anurans, one salamander (tailed amphibian) and 5 caecilians. Small but significant contributions came in long intervals from different taxonomist from time to time. Finally, Das and Dutta (1998) consolidated the list comprising of 219

species of amphibians in the country. Although India has a very rich fauna of Amphibians (Inger and Dutta, 1987; Das, 1996), extensive quantitative ecological studies have not been made on the amphibian communities in the Indian ecosystems except for the work of Dash and Mahanta (1993).

Recent review of literature on the new species and new reports of Ranids from South East Asia includes the following. Voris and Inger (1995) reported 9 species of ranidae namely; *Amolops phaeomerus*, *A. poecilus*, *Rana blythi*, *R. ibanorum*, *R. ingeri*, *R. kuhli*, *R. hosei*, *R. chalconota* and *R. signatae* out of the total 19 frog species collected while studying the abundance of frogs along streams in Bornean Forest. Inger and Chanard (1997) described a new species of Ranid frog from Thailand (*Rana archotaphus*) with comments on *Rana livida*. Zug *et al.*, (1998) reported 8 species of Ranidae (*Occidozyga lima*, *Rana lateralis*, 2 *Rana limnocharis* complex, *Rana macrodactyla*, *Rana rugulosa*, *Rana tigerina* and *Tomopterna breviceps*) out of 16 species of amphibians surveyed on the herpetofauna of Chatthin Wildlife Sanctuary, Myanmar. Das (1998) described a new species *Rana charlesdarwini* from Mount Harriet National Park, South Andaman Island. Zainuddin (1999) reported a total of 18 species with 144 individuals of anurans occurred in Bario and vicinity which was dominated by the family of Ranidae with 72% out of the total of the individuals captured. Liu *et al.*, (2000) described a new species of stream-breeding frog of the genus, *Amolops bellulus* from Western Yunnan, China. Bain *et al.*, (2003) described 6 new species of the genus *Rana*; *Rana bacboensis*, *R. daorum*, *R. hmongorum*, *R. morafkai*, *R. banaorum* and *R. megatympanum* and also made taxonomic revisions on cryptic species of a cascade frog from Southeast Asia. Orlov *et al.*, (2003) described a new species of cascade

frog, *Rana trankieni* from North Vietnam. Bain and Troung (2004) gave new descriptions of two new species, *Rana iriodes* and *Rana tabaca* and did studies on the herpetofaunal diversity of Ha Giang Province in Northeastern Vietnam. Bain and Stuart (2005) described a new species of cascade frog, *Rana indeprensa* belonging to the *Rana livida* species complex from Nakhon Ratchasima and Nakhon Nayok Provinces, eastern Thailand and gave new data on *Rana banaorum* and *R. morafkai*. Stuart and Chan-ard (2005) described two new species, *Huia absita* and *Huia melasma* from Xe Sap National Biodiversity Conservation Area, southern Laos and Tham Tarn Lot (= Chalerm Rattanakosin) National Park and Kaeng Krachan National Park, western Thailand respectively. Bain *et al.*, (2006) described three new Indochinese species of cascade frogs (Amphibia: Ranidae) allied to *Rana archotaphus*. Matsui and Jaafar (2006) described a new species of cascade frog of the genus *Rana*, *Rana monjerai* from west Malaysia. Matsui and Nabhitabhata (2006) reported a new species of torrent-dwelling ranid frog of the genus *Amolops*, *Amolops panhai* from western to Peninsular Thailand. Orlov *et al.*, (2006) described a new frog species, *Rana gigatympana* of the family Ranidae (cascade frog from *Odorrana*-group) from central Vietnam. Rao and Wilkinson (2007) described a new species *Amolops caelumnoctis* from a mountainous area of southern Yunnan Province, China.

Northeast (NE) India is situated at the confluence of the Indo-Malayan, Indo-Chinese and Indian biogeographical regions. This unique position, coupled with its physiography, has laid a foundation for the proliferation of a variety of habitats, which harbour a diverse biota with a high level of endemism (Mani, 1974). As many as 54 species under 6 families and 18 genera have been found to occur in Northeast

India (Chanda, 1994). Sen (2004) recorded and reported 83 species of amphibian comprising 78 anurans, 4 gymnophiona and 1 caudata. She reported 78 species of anurans belong to 25 genera, 6 families and 1 order reported from the Northeast India and the state of Mizoram is represented by 19 species of amphibians belonging to 4 families and 1 order. Over 6000 species of amphibians are known to occur around the globe. So far, 286 species of them are known from India and more than 105 species are found in the North East India (Ahmed *et al.*, 2009).

The first record of frogs belonging to the family Ranidae from Northeast India have been recorded by several workers: *Rana alticola* and *Rana laticeps* were originally recorded from Khasi hills, Meghalaya (Boulenger, 1882); *Rana assamensis* was erected on the basis of single specimen collected from Khasi hills, Meghalaya (Sclater, 1892); *Rana gerbillus* was reported from “Yumbung” Abor foot hills, Arunachal Pradesh (Annandale, 1912); *Rana garoensis* was described from Garo hills, Meghalaya (Boulenger, 1920); *Rana danieli* and *Rana mawphlangensis* were originally recorded from Mawphlang, Khasi hills, Meghalaya (Pillai & Chanda, 1977); *Rana bilineata* is known only from the Holotype which was erected on a single young frog collected from Garo hills, Meghalaya, Northeast India (Pillai & Chanda, 1981); Kiyasetuo and Khare (1986a) described a new genus of frog *Pterorana khare* (Ranidae) from Nagaland. Since the original description of the species in 1986, male specimens have been reported from various parts of north-east India: Arunachal Pradesh (Chanda, 1994; Pawar and Birand, 2001); Nagaland (Ao *et al.*, 2003); bank of river Tlawng, Sairang (23°36’N, 93°00’E; alt. 2000-2500 m), 21 km from Aizawl, Mizoram (Dey and Ramanujam, 2003); and Dhaleswari river, Bairabi, Mizoram (Sen and Matthew, 2003). Ao *et al.*, (2003) gave the range

extension in Nagaland as Rukhroma (alt.1440 m) and Jokhoma (alt. 1600 m). *Rana mawlyndipi* has been recorded originally from Meghalaya (Chanda 1990a); *Rana ghoshi* has been described for the first time from Manipur (Chanda, 1990b); *Hoplobatrachus crassus* from North eastern region in Assam and Arunachal Pradesh (Bordoloi and Borah, 1999), *Paa annandalii* from Arunachal Pradesh (Bordoloi *et al.*, 2001; Bordoloi and Borah, 2001; Borah and Bordoloi, 2001), *Euphlyctis hexadactylus* from north east India (Sen and Matthew, 2004), *Rana chloronota* from Mizoram, North-Eastern India (Lalremsanga *et al.*, 2007a). First report of *Amolops kaulbacki* from India (Sailo *et al.*, 2007). Record of *Sylvirana leptoglossa* (from Kolasib district, Mizoram. Northeastern India (Lalremsanga *et al.*, 2007b). First record of *Pterorana khare* from Meghalaya, East Khasi Hills District (Rangad *et al.*, 2007), *Ingerana borealis* (Annandale, 1912): a new record from Mizoram (India), with notes on its systematic position and natural history (Sailo *et al.*, 2009). *Amolops nidorbellus* and *Amolops kohimaensis* from Nagaland, North East India (Biju *et al.*, 2010). Studies on little known Amphibian species of North East India by Mathew and Sen (2009), Amphibians and Reptiles of Northeast India: A photographic guide by Ahmed *et al.*, (2009) are some examples.

Rana alticola Boulenger, 1882, was described on the basis of four specimens from Shillong (Khasi Hills District, Meghalaya State, India) under the name *Hylorana pipiens* Jerdon, 1870. This is a poorly studied species distributed widely in latitude, longitude and elevation, from the Hills of Assam (India) to southern Thailand. Although adults are rare in herpetological collections, the tadpoles have been known for a long time (Boulenger, 1882; Annandale, 1912; Smith, 1924a, b; review by Bourret, 1942). Boulenger (1920) in his book “A monograph of the South

Asian, Papuan, Melanesian and Australian frogs of the genus *Rana*” mentioned the males of *Rana alticola* with internal vocal sacs and no other secondary sexual characters. The only publications on *Rana alticola* are those by Sahu and Khare (1980) where they gave field key of *Rana alticola* tadpoles; Sahu and Khare (1988) on the food and feeding habits of *Rana alticola* during different stages of metamorphosis and also by Grojean *et al.*, (2003) in which they gave the morphology and buccopharyngeal anatomy of the tadpole of *Rana (Nasirana) alticola*.

From the literature surveyed, it is evident that there is scarcity of information on the distribution of *Rana alticola* in North east India. Hence, this chapter deals with the distribution of *Rana alticola* in Mizoram, with its descriptions and morphometric measurements.

MATERIALS AND METHODS

To know the distribution of *Rana alticola* in Mizoram, survey was conducted from the year 2005. Observations were made at different locations within Mizoram. The locations and elevations of the areas where *Rana alticola* was found were determined with the help of a Global Positioning System (GPS) (Garmin Company). Standard survey techniques for amphibians including anuran calling surveys, egg mass surveys, larval surveys, and visual encounter surveys for adults were used. Specimens from different localities of Mizoram were collected. Extensive survey was made during the day and night. During the night time, study sites were surveyed with the help of head lamps, torch light, bamboo torch (locally made torch where kerosene is poured inside the hollow bamboo stumps which is then plugged with a cloth and then burned) and dip-net as well as mosquito net for larvae. The adults were captured by hand or net and photographed in live condition. After taking photographs and identification, some of the animals were released back to their natural environment and some were preserved in 4 % formaldehyde for further studies. Eggs and tadpoles collected from their natural environment were reared to metamorphosis for species confirmation by maintaining in a plastic tray containing stream or pond water in the laboratory condition. Collections were made from different habitats such as forest covers, rivers, streams, ponds, rain fed pools, open fields with vegetation, etc.

Description of the specimen was noted down in the morphometric data sheet, which is incorporated as the following:

Specimen No.:

Museum No.:

Scientific Name:

Place:

| | |
|-----------------------|--------------------|
| Habitat: | Microhabitat: |
| Temperature: | Relative humidity: |
| Rainfall: | Colour: |
| Stage: Juvenile/Adult | Sex: |
| Date: | Time: |
| Sky: Cloudy/Clear | Elevation: |
| GPS Location: | |

After making careful observations, the collected specimens were identified; morphometric measurements were taken as shown in the morphometric data sheet. Measurements of the frogs were carried out using a dial caliper (Mitutoyo series No. 505-671). While some of the frogs were released back, few were sacrificed by anesthetization with chloretone / chlorobutanol solution and then fixed in 4 % formaldehyde for preservation, but before keeping in formaldehyde, a small incision was made on the lateral side of the abdomen for proper preservation. The tadpoles were euthanized with MS222 solution and preserved in 4% formaldehyde. The preserved specimens were then identified with the help of the monograph key prepared by Chanda (1994), as well as with the help of Zoological Survey of India, Shillong and other literatures. Voucher specimens of species have been lodged at the Developmental Biology Laboratory, Department of Zoology, NEHU and Zoological Survey of India, Shillong. Specimens were sexed either according to their external characters (in the case of adult breeding males) or through a slight lateral incision in order to examine the gonads. Morphometric measurements largely follow the combination of Chanda (1994), Bain *et al.*, (2006) and Ohler (2007).

Abbreviations used are as follows:

1. SVL: From tip of snout to the vent distance
2. SL: From eye to snout tip distance
3. EN: From eye to nostril distance
4. NS: From nostril to snout distance
5. ET: From eye to tympanum distance
6. INS: Distance (interspaced) between nostrils
7. IOS: Distance (interspaced) between eyes
8. HTYD: Horizontal tympanum distance
9. VTYD: Vertical tympanum distance
10. HL: Head length-distance from tip of snout to angle of jaws
11. HWN: Head width-width of the head at nostril
12. HWAE: Head width-width of the head at anterior eye
13. HWPE: Head width-width of the head at posterior eye
14. HWAJ: Head width-width of the head at the angle of the jaws
15. HDN: Head depth-depth of the head at nostril
16. HDE: Head depth-depth of the head at eye
17. HDAJ: Head depth-depth of the head at the angle of the jaws
18. IAE: Distance between anterior eyes
19. IPE: Distance between posterior eyes
20. AG: Distance from armpit to groin
21. FLL: From the insertion of the forelimb to the tip of the third finger
22. Hnd. L: Hand length (From the base of the outer palmar tubercle to the tip of the finger)

23. F1: Distance from base of the inner metacarpal tubercles (IMC) to the tip of the first finger
24. F2: Distance from base of the (IMC) to the tip of the second finger
25. F3: Distance from base of the (IMC) to the tip of the third finger
26. F4: Distance from base of the (IMC) to the tip of the fourth finger
27. HLL: Length of hind limbs from its insertion to the tip of the longest toe
28. TBL: Tibia length
29. TBW: Tibia width-maximum width of the tibia
30. TL: Tarsus length
31. Ft. L: Foot length (From proximal edge of inner metatarsal tubercle to tip of fourth toe)
32. T1: Distance from base of metatarsal tubercles to tip of first toe
33. T2: Distance from base of metatarsal tubercles to tip of second toe
34. T3: Distance from base of metatarsal tubercles to tip of third toe
35. T4: Distance from base of metatarsal tubercles to tip of fourth toe
36. T5: Distance from base of metatarsal tubercles to tip of fifth toe

RESULTS

Area surveyed: During the present investigation, Mizoram which is in the southernmost state in North-east India, and lies between 21°56'N – 24°31'N and 92°16'E – 93°26'E has been surveyed for the distribution of *Rana alticola*. The terrain is a geologically distinctive mountainous area with precipitous slopes forming deep gorges with streams and rivers in between. Almost all the hill ranges are oriented in a north-south direction. Phawngpui (or Blue Mountain) is the highest peak at 2157 m and the lowest place is Bairabi at 40 m above mean sea level. The average height of hill ranges in Mizoram is 920 m. The most important and useful rivers are the Tlawng (also known as Dhaleswari or Katakhal), Tut (Gutur), Tural (Sonai) and Tuivawl which flows through the northern territory and eventually join river Barak in Cachar. The Chhimtuipui River (Kolodyne) which originates in Myanmar is an important river in the south Mizoram. It has four tributaries and the river is in patches. The western part is drained by Khawthlangtuipui (Karnaphuli) and its tributaries. Lakes are scattered all over the state and important of them are Palak, Tamdil, Rungdil, and Rengdil.

During the study period i.e. 2005 to 2007, different parts of Mizoram were surveyed. Eight districts i.e. Aizawl, Kolasib, Champhai, Mamit, Serchhip, Lunglei, Lawngtlai and Saiha Districts were surveyed for studying the distribution of *Rana alticola* (Table 1.1; Fig 1.1). Extensive survey and collections have been made from the following localities:

1) Aizawl District:

Aizawl: Aizawl, the capital of Mizoram lies between 23°43'N and 92°40'E at an altitude of 743-965 m asl. Different parts of Aizawl town where water bodies are

found were surveyed. *Rana alticola* tadpoles were encountered at Chite River which flows at the heart of Aizawl. The tadpoles were collected during the month of October. But subsequent survey does not yield any adults (Table 1.1; Fig 1.2a).

Rungdil: Rungdil is a lake which means lake of partridge. It acquired this name as one time it was the habitat of a large number of partridges. It consists of two lakes, more or less alike, separated by a narrow stretch of land about 10 m. It is situated 14 km from Suangpuilawn village in Aizawl district and is 127 km from Aizawl city. It has an area of 2.5 hectares. These two lakes lies between 23°59'N and 93°00'E at an elevation of about 332 m asl. *Rana alticola* tadpoles and adults were collected from this area. The adults were collected during the months of July and August whereas the tadpoles were collected during the months of September to December and January (Table 1.1; Fig 1.2b).

Sairang: Sairang is an important town situated at Aizawl district. It is about 25 km from Aizawl city. It lies between 23°48'N and 92°37'E at an altitude of 50-80 m asl. The Tlawng River, which is the longest river in Mizoram pass through this town from south to north direction. A large number of sand mining is going on in this river and as a result, there is disturbance of the aquatic fauna present here. However, a large number of *Rana alticola* tadpoles are present here and the adults are also present. The tadpoles were collected during the months of September to December and from January to March. The adults were caught in the months of August, September and October (Table 1.1; Fig 1.2c).

Sihhmui: Sihhmui is a town located at Aizawl District which is about 27 km from Aizawl city. It is located between 23°47'N and 92°39'E at an altitude of 180 m asl. *Rana alticola* tadpoles were found along the streams but adults were not collected.

Tadpoles were collected during the months of September to December and January (Table 1.1; Fig 1.2d).

Tamdil: Tamdil Lake is the second largest lake in Mizoram. It lies between 23^o44'N and 92^o31'E, the elevation of this study site is 760 m asl. It is about 110 km from Aizawl and it has been identified as wetland under National wetland conservation programme 2006 – 2007 by the Government of India. Today the lake is an important tourist attraction and a holiday resort. A large number of *Rana alticola* tadpoles and adults were found here. Tadpoles were collected from the months of July to December and from January to March. Adults were collected from the months of June to October (Table 1.1; Fig 1.2e).

Tuirial: Tuirial is a river and it lies between 23^o43'N and 92^o47'E at an elevation of 179 m asl. It is about 27 km from Aizawl. The stream bed is sandy with rocks and huge boulders, shallow or temporarily deep in some areas. A large number of *Rana alticola* tadpoles are found here during the months of September to December and also in the month of January and February. No adults were found here (Table 1.1; Fig 1.2f).

Tuirini: Tuirini is a river and lies between 23^o41'N and 92^o53'E at an elevation of 298 m asl. It is located at about 56 km from Aizawl. The river flows through cultivated lands, paddy fields and forest area and later joins Tuirial after proceeding about 45 km towards north. Both adults and tadpoles of *Rana alticola* are present here. Adults were collected in the months of August and September. Tadpoles were collected during the months of August to December and January (Table 1.1; Fig 1.2g).

2) Kolasib District:

Buhchang: Buhchang is a village flanked by paddy fields and fish ponds, and lies between 24°20'N and 92°39'E at an elevation of 46 m asl. It is situated in Kolasib district. Both the adults and tadpoles of *Rana alticola* were encountered and tadpoles were found in a stream near the paddy fields. Adults were collected during the month of September. Tadpoles were collected during the months of September to December and January (Table 1.1; Fig 1.3a).

Herhse: Herhse is a stream which is one of the tributaries of Tuitun River, one of the major rivers in Mizoram. It lies between 23°58'N and 92°41'E. This stream is covered with thick forests which make it very ideal for amphibian study. The stream bed is mainly composed of rocks and pebbles. The elevation is about 308-326 m asl and is situated about 60 km from Aizawl. Both adults and tadpoles of *Rana alticola* are present here. The adults were collected in the months of June to October. Tadpoles were collected during the months of August to December and from January to March (Table 1.1; Fig 1.3b).

Kawnpui: Kawnpui is a town located in Kolasib district and lies within 23°56'N and 92°41'E at an altitude of 910 m asl. It is about 69 km from Aizawl. Different streams and ponds in this area were surveyed. *Rana alticola* tadpoles were collected from this place during the months of September and October. No adults were seen (Table 1.1; Fig 1.3c).

3) Champhai District:

Lundil: Lundil is a lake situated at Kawlkulh village which is in Champhai district. It lies between 23°43'N and 93°05'E at an altitude of 965 m asl. It is about 130 km

from Aizawl. *Rana alticola* tadpoles and adults were collected from this place during the months of August and September (Table 1.1; Fig 1.4).

4) Mamit District:

Lengpui: Lengpui is a town in Mamit District where the only airport in Mizoram known as ‘Lengpui Airport’ is situated. It lies between 23°49'N and 92°37'E at an elevation of 390 – 400 m asl. Different water bodies were surveyed and only the tadpoles of *Rana alticola* were found here. The tadpoles were collected in the months of August to December and January. No adults were collected (Table 1.1; Fig 1.5a).

Tut: Tut is a river located at Mamit district and lies between 23°46'N and 92°31'E at an altitude of 74 m asl. The river originates from Lunglei district and flows south to north direction. This place is about 76 km from Aizawl. A number of streams and thick forest covers are present which gives an ideal habitat for anurans. The adults were collected from this river during the month of September. A large number of *Rana alticola* tadpoles are found in this river. The tadpoles were collected during the months of September to December and in the months of January and February (Table 1.1; Fig 1.5b).

5) Serchhip District:

Chhingchhip: Chhingchhip is a village which lies between 23°27'N and 92°51'E at an elevation of 1113 m asl in the Serchhip district. It is about 82 km from Aizawl. Different ponds and streams were surveyed. Both tadpoles and adults of *Rana alticola* were collected in the months of August and September (Table 1.1; Fig 1.6a).

Mat: Mat is a river which is located between 23°27'N and 92°50'E at an altitude of 651 m, in the Serchhip district, which is about 90 km from Aizawl. This river

originates from the central part of Aizawl district and flows north to south, crossing Serchhip district and later confluent with Chhimtuipui River at the district boundary between Lunglei district and Saiha district. A large number of *Rana alticola* tadpoles were found in this river during the months of October to December and January. No adults were found here (Table 1.1; Fig 1.6b).

Thenzawl: Thenzawl is a town located in the Serchhip district and lies between 23°17'N and 92°46'E at an elevation between 741-810 m asl. It is about 120 km from Aizawl. The terrain of Thenzawl area is plain and most of the area is utilized for plantation and fishery. Different water bodies were surveyed. Only tadpoles of *Rana alticola* were encountered in the months of September to December and January (Table 1.1; Fig 1.6c).

6) Lunglei District:

Theiriat: Theiriat is located in the Lunglei district and lies between 22°55'N and 92°45'E at an elevation between 1048-1060 m asl. It is about 225 km from Aizawl. Both adults and tadpoles of *Rana alticola* are collected from this area. The adults and tadpoles were collected during the months of August and September (Table 1.1; Fig 1.7).

7) Lawngtlai District:

Lawngtlai: Lawngtlai is the Capital of Lawngtlai district located between 22°31'N and 92°53'E at an elevation of 847 m asl. It is about 296 km from Aizawl. Both adults and tadpoles of *Rana alticola* were collected from the surrounding streams and ponds. The adults and tadpoles were collected during the month of September (Table 1.1; Fig 1.8).

8) Saiha District:

Khankawn: Khankawn is a town located between 22°22'N and 92°57'E at an altitude of 193 m asl in the Saiha district. It is about 370 km from Aizawl. *Rana alticola* tadpoles were collected from different water bodies. No adults were collected but a large number of tadpoles were collected during the month of September (Table 1.1; Fig 1.9a).

New Latawh: New Latawh is also a small town located at Saiha District near the Chhimtuipui River. It is about 340 km from Aizawl and lies between 22°22'N and 92°55'E at an altitude of 458 m asl. The village is surrounded by thick forest covers and a few streams run near the edges of these forests. A large number of *Rana alticola* tadpoles and adults are found here during the month of September (Table 1.1; Fig 1.9b).

Three survey sites namely Sairang River, Herhse stream and Tamdil Lake were selected for the study sites and designated as study sites I, II and III respectively. These three sites were selected as both the adults and tadpoles of *Rana alticola* were present and also they were ideal for studying the breeding behaviour in the field and its natural habitat (Fig 2.1).

Adults of *Rana alticola* were collected from both permanent ponds and streams and rivers covered by surrounding vegetations and among bushes surrounding water bodies during its breeding period. The tadpoles were usually found to inhabit a part of water where the current is slow and are usually found in large number. In the present survey, tadpoles were collected from all the 20 survey sites which are Aizawl, Rung dil, Sairang, Sihhmui, Tam dil, Tuirial, Tuirini, Buhchang, Herhse, Kawnpui, Lun dil, Lengpui, Tut, Chhingchhip, Mat, Thenzawl,

Theiriati, Lawngtlai, Khankawn and New Latawh but adults were encountered only from 12 survey sites namely Rungdil, Sairang, Tamdil, Tuirini, Herhse, Buhchang, Lundil, Tut, Chhingchhip, Theiriati, Lawngtlai, and New Latawh (Table 1.1). During the present survey, tadpoles of *Rana alticola* were encountered during the months of August, September, October, November, December, January, February and March. The froglets were found in plenty during the months of April and May. No tadpoles were found in the months of May, June and July.

Description of the species:

Adult:

Diagnosis: Dorsally brown to orange in female (Fig. 1.10) and yellowish to light brown in male (Fig. 1.11a). A feebly prominent, narrow, glandular dorsolateral fold running from posterior region of eyes and above the tympanum to hip. Ventrally almost white in colour. Skin is smooth. A feebly prominent glandular lateral fold present. Another glandular fold running from the posterior region of tympanum to shoulder. Presence of white line mid ventrally at the throat region extending up to the angle of jaw in both male and female (Fig 1.11b).

Description: Head longer than broad, depressed; snout slightly longer than eyes, obtusely pointed, projecting slightly beyond mouth; canthus rostralis distinct; loreal region concave; internarial space slightly less than interorbital width, which is slightly less than eye; nostrils much closer to tip of snout than to eyes; tympanum very distinct, dark brown to reddish brown in colour, two-thirds of eyes, separated from the latter by a space about half of tympanic diameter; vomerine teeth oblique in position between the choanae, equidistant from each other and choanae (Fig 1.12). Tongue bifid (Fig 1.13).

Forelimbs short; fingers long, slender free with horse-shoe shaped discs, separating upper from the lower surface; relative length of fingers: $II < I < IV < III$; first finger slightly longer than second, third longest, much longer than snout; subarticular tubercles moderately large and prominent (Fig 1.14).

Hindlimbs long; slender; tibiotarsal articulation reaching beyond tip of snout; heels strongly overlapping when hindlimbs folded at right angles to body; tibia five to six times as long as broad, more than half of the length of snout to vent; toes having small discs like fingers but slightly broader than the latter; two phalanges of fourth toe free; subarticular tubercles moderately large and prominent; outer metatarsals separated at the base; inner metatarsal tubercle small, oval and one-fourth of inner toe; a small indistinct outer metatarsal tubercle present. Relative length of toes: $I < II < III < V < IV$ (Fig 1.15).

Webbing formula: **I** $_{1-1\frac{1}{2}}$ **II** $_{1-2}$ **III** $_{1-2}$ **IV** $_{2-1}$ **V** (Fig 1.15)

Sexual dimorphism: Female larger than male (Fig 1.16). The Snout Vent Length (SVL) of females ranges from 41.9 – 60.92 (51.33 ± 4.25 ; N=40) (Table 1.2; Fig 1.10) whereas the SVL of males ranges from 32.33 - 46.89 (39.08 ± 3.27 ; N=40) (Table 1.2; Fig 1.16). No other sexual dimorphism seen. Nuptial pad is absent during the breeding season and vocal sac is internal.

Larval morphology:

In dorsal view, the body is elliptical, widest at the posterior third; snout semi-circular. In profile, body depressed; snout rounded. Eyes are slightly bulging, directed almost laterally and positioned dorsolaterally, not visible in ventral view. Nares 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 single, sinistral, bulb shaped, attached to body wall except at its extremity, positioned ventrolaterally, oriented more horizontally than posterodorsally. Spiracle opening is oval. Tail musculature robust, gradually tapering, almost reaching tail tip. Tail fin moderately high, not extending on to body. Upper fin slightly higher than lower. Oral disk large, anteroventral, slightly emarginated, directed ventrally. There is presence of a pair of parotoid glands behind the eyes. There is also presence of a pair of pectoral gland, a pair of posteroventral gland, supracaudal gland and infracaudal gland (Fig 1.17). The colour of the tadpole 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 dark brown grey in colour. Caudal muscle sandy transparent with small black spots and an ocellus at the base with a red halo (Fig 1.18)

DISCUSSION

The present investigation revealed the current status on the distribution of *Rana alticola* in different parts of Mizoram. Earlier works reported the distribution of *Rana alticola* in India from states like Meghalaya, Assam, Tripura, West Bengal, Orissa and Andaman, also from other countries like Sri Lanka, Nepal, China, Japan, Indonesia and Malaysia (Chanda, 1994). There have been only few reports on the occurrence of *Rana alticola* from Mizoram. Pawar and Birand (2001) reported its occurrence from Ngenpui Wildlife Sanctuary situated in the Saiha District of Mizoram and also by Sen (2004) without mentioning the location. However, in the present investigation, extensive survey conducted throughout the state of Mizoram provided more information on the distribution of *Rana alticola* from twelve more collection sites in Mizoram. The collections of both the adults and tadpoles were made from all the eight districts of Mizoram namely Aizawl, Kolasib, Champhai, Mamit, Serchhip, Lunglei, Lawngtlai and Saiha Districts and the altitude of the collection sites range from 46 m asl at Buhchang, Kolasib District, to 1113 m asl from Chhingchhip located at Serchhip District, indicating that *Rana alticola* has a wide range of distribution and that it is adapted to low and high altitude area. Although Sahu and Khare (1980) mentioned *Rana alticola* as one of the high altitude frog, however Ahmed *et al.*, (2009) reported its presence from 40-100 m without mentioning the exact location. Among anurans, generally there is a decrease in the number of species with altitude, although local environmental conditions may alter this gradient (Duellman and Trueb, 1994). Many factors have been suggested to influence patterns of species distribution and assemblage composition at specific

sites, such as competition (Morin 1983), predation (Gascon, 1991; Eterovick and Sazima, 2000), morphological and behavioural attributes (Crump 1974; Toft 1985)

During the present survey, it was found that *Rana alticola* was collected from both permanent ponds, streams and rivers covered by surrounding vegetations and among bushes surrounding water bodies during its breeding period as also seen in other ranids like green frog, *R. clamitans*; bullfrogs, *R. Catesbeiana* which select quiescent water of permanent ponds (U.S. EPA. 2002). The choice of specific microhabitats is related to morphological, physiological and behavioural adaptations of species (Crump 1971; Pough *et al.*, 1977; Cardoso *et al.*, 1989), and species with broad niches are expected to be more widespread because they may tolerate a greater variety of habitat conditions (Gaston *et al.*, 1997; Pyron 1999). Heterogeneous habitats also favour an increase in species richness, since a higher combination of microhabitat types and ecological niches is available (MacArthur 1968).

Earlier workers mentioned that although adults are rare in herpetological collections, the tadpoles have been known for a long time (Boulenger, 1882; Annandale, 1912; Smith, 1924a, b). Similarly, Chanda (1994) mentioned that the adults of *Rana alticola* are extremely rare and nocturnal but their tadpoles are abundantly available. This is also true for the present survey where tadpoles are encountered in all the 20 survey sites like Aizawl, Rung dil, Sairang, Sihhmui, Tam dil, Tuirial, Tuirini, Herhse, Kawnpui, Buhchang, Lun dil, Tut, Lengpui, Chhingchhip, Mat, Thenzawl, Theiriat, Lawngtlai, Khankawn and New Latawh whereas adults are encountered only in 12 survey sites namely Rungdil, Sairang, Tamdil, Tuirini, Herhse, Lundil, Tut, Chhingchhip, Thenzawl, Theiriat, Lawngtlai, and New Latawh (Fig 1.1). Physical conditions of an organism's environment,

temperature, light, moisture – and the food resources it contains primarily determine the distribution of the organism in space and time. These environmental factors are however far from evenly distributed, resulting in uneven distribution of the organisms themselves (Cox and Moore, 1980). The investigation conducted to know the distribution of this species in Mizoram shows that *Rana alticola* tadpoles are adapted to diverse habitats where they are present in all the survey sites.

The tadpoles of *Rana alticola* in Mizoram are usually found to inhabit a part of water where the current is slow and they are usually found in large number. Sahu and Khare (1980) reported that the tadpoles are generally found in the coves of mountain torrents where they usually gaze in large number. Grosjean *et al.*, (2003) also collected the tadpoles from a large pool under a waterfall of a river (about 10 m wide) running through a primary forest but reported that the tadpoles did not gather in schools, and stayed still on the substrate where they were conspicuous due to their black coloration and large size.

During the survey, tadpoles of *Rana alticola* were encountered during the months of August, September, October, November, December, January, February and March. The froglets were found in plenty during the months of April and May. No tadpoles were found in the months of May, June and July. It may be suggested that the tadpoles have metamorphosed into froglets. It may be mentioned that Sahu and Khare (1980) reported the occurrence of the tadpoles in almost all months of the year. However, Inthara *et al.*, (2005) recorded samples of tadpoles of *Rana alticola* from Thailand during the months of January, March, May, September, October and December as observed in the present study in Mizoram. Tadpoles from different localities referred to *Rana alticola* have been described by previous authors from

northeastern India [Abor, Arunachal Pradesh (Annandale, 1912), Shillong, Khasi Hills (Annandale in Smith, 1924b) and northeastern hill region (Sahu & Khare, 1980)], peninsular Thailand (Smith, 1924a) and Ramon Forest Park, Phang Nga Province, Thailand (Grosgean *et al.*, 2003).

The present study reveals that adults of *Rana alticola* were found in a large number from all the study sites during the breeding season which starts from late June to early October. The adults were only occasionally encountered, but the distinctive tadpoles are impossible to overlook. Indications are that the species is not uncommon in areas where it occurs. *Rana alticola* has been listed as Least Concern in view of its wide distribution, presumed large population, and because it is unlikely to be declining fast enough to qualify for listing in a more threatened category (van Dijk, P.P *et al.*, 2004). As seen from the previous works, it is evident that there is scanty information on the adults of *Rana alticola*. This is a poorly studied species distributed widely in latitude, longitude and elevation, from the Hills of Assam (India) to southern Thailand (Grosgean *et al.*, 2003).

Table 1.1: Survey sites of *Rana alticola*.

| Sl. No. | Districts | Survey sites | Latitudes | Longitudes | Elevation (m asl) | Adults | Tadpoles |
|---------|-----------|--------------|-----------|------------|-------------------|--------|----------|
| 1. | Aizawl | Aizawl | 23°43'N | 92° 40'E | 743-965 | - | + |
| | | Rung dil | 23°59'N | 93°00'E | 332 | + | + |
| | | Sairang | 23°48'N | 92°37'E | 50-80 | + | + |
| | | Sihhmui | 23°47'N | 92°39'E | 184 | - | + |
| | | Tam dil | 23°44'N | 92°57'E | 745 | + | + |
| | | Tuirial | 23°43'N | 92°47'E | 179 | - | + |
| 2. | Kolasib | Tuirini | 23°41'N | 92°53'E | 298 | + | + |
| | | Buhchang | 24°20'N | 92°39'E | 46 | + | + |
| | | Herhse | 23°58'N | 92°41'E | 308-326 | + | + |
| | | Kawnpui | 23°56'N | 92°41'E | 910 | - | + |
| | | Lun dil | 23°43'N | 93°05'E | 965 | + | + |
| | | Lengpui | 23°49'N | 92°37'E | 390-400 | - | + |
| 3. | Mamit | Tut | 23°46'N | 92°31'E | 74 | + | + |
| | | Chhingchhip | 23°27'N | 92°51'E | 1113 | + | + |
| | | Mat | 23°27'N | 92°50'E | 651 | - | + |
| 4. | Serehhip | Thenzawl | 23°17'N | 92°46'E | 741-810 | - | + |
| | | Theiriat | 22°55'N | 92°45'E | 1048-1060 | + | + |
| 5. | Lunglei | Lawngtlai | 22°31'N | 92°53'E | 847 | + | + |
| | | Satha | 22°22'N | 92°57'E | 193 | - | + |
| 6. | Lawngtlai | New Latawh | 22°22'N | 92°55'E | 458 | + | + |

Table 1.2: Morphometric measurements of male and female *Rana alticola*
(N=Total number of frogs examined)

| Sl No | Characters | Male | Female | Sl. No. | Characters | Male | Female |
|-------|------------|-------------------------------|-------------------------------|---------|------------|-------------------------------|--------------------------------|
| | | N=40 | N=40 | | | N=40 | N=40 |
| 1 | SVL | 39.08 ± 3.27 32.33 - 46.89 | 51.33 ± 4.25 41.9 - 60.92 | 19 | IPE | 9.47 ± 0.83 8.2 - 10.94 | 13.54 ± 0.69 12.4 - 15.24 |
| 2 | SL | 6.83 ± 0.69 5.82 - 7.88 | 9.34 ± 0.84 7.34 - 10.58 | 20 | AG | 17.92 ± 2.84 11.36 - 23.34 | 29.77 ± 3.10 25.51 - 35.12 |
| 3 | EN | 4.11 ± 0.44 3.16 - 4.75 | 5.71 ± 0.56 4.66 - 6.7 | 21 | FLL | 24.36 ± 1.71 22.34 - 27.72 | 37.47 ± 2.09 33.62 - 40.18 |
| 4 | NS | 2.87 ± 0.51 2.28 - 4.26 | 3.63 ± 0.41 2.68 - 4.26 | 22 | Hnd.L | 11.11 ± 0.8 9.94 - 12.8 | 15.53 ± 0.81 14.32 - 17.36 |
| 5 | ET | 1.23 ± 0.29 0.78 - 1.68 | 2.12 ± 0.34 1.56 - 2.8 | 23 | F1 | 7.20 ± 0.73 6.06 - 8.48 | 10.38 ± 0.88 9.4 - 12.52 |
| 6 | INS | 3.19 ± 0.32 2.68 - 3.64 | 4.18 ± 0.22 3.7 - 4.92 | 24 | F2 | 6.91 ± 0.67 5.76 - 7.84 | 10.09 ± 0.77 9.3 - 11.58 |
| 7 | IOS | 4.54 ± 0.43 3.82 - 5.24 | 6.00 ± 0.40 5.5 - 6.88 | 25 | F3 | 11.11 ± 0.8 9.94 - 12.8 | 15.53 ± 0.81 14.32 - 17.36 |
| 8 | HTYD | 3.88 ± 0.33 3.26 - 4.64 | 4.19 ± 0.45 3.44 - 5.34 | 26 | F4 | 9.15 ± 0.91 7.76 - 10.6 | 13.13 ± 0.93 11.66 - 14.72 |
| 9 | VTYD | 3.26 ± 0.28 2.8 - 3.9 | 3.91 ± 0.40 3.12 - 4.5 | 27 | HLL | 66.34 ± 4.25 60.4 - 79.34 | 96.37 ± 4.17 90.56 - 103.75 |
| 10 | HL | 12.98 ± 0.76 11.96 - 14.56 | 18.91 ± 1.19 16.94 - 20.65 | 28 | TBL | 22.64 ± 1.24 20.96 - 26 | 32.14 ± 1.79 29.72 - 35.68 |
| 11 | HWN | 5.16 ± 0.42 4.68 - 5.78 | 7.15 ± 0.86 6.13 - 9 | 29 | TBW | 3.53 ± 0.40 2.84 - 4.36 | 4.82 ± 0.47 4 - 5.48 |
| 12 | HWAE | 8.37 ± 0.52 7.78 - 9.68 | 11.82 ± 1.0 10.42 - 12.6 | 30 | Tarsus L | 12.2 ± 0.71 11.4 - 12.82 | 17.44 ± 1.34 14.12 - 19.67 |
| 13 | HWPE | 10.86 ± 0.87 9.98 - 11.86 | 15.77 ± 0.74 14.35 - 16.92 | 31 | Ft.L | 17.69 ± 1.63 14.8 - 20.54 | 26.06 ± 1.35 23.02 - 28.52 |
| 14 | HWAJ | 11.58 ± 0.64 10.84 - 13.7 | 16.96 ± 0.89 15.91 - 18.04 | 32 | T1 | 5.98 ± 0.75 5 - 7.84 | 8.52 ± 0.79 7.1 - 9.56 |
| 15 | HDN | 3.90 ± 0.50 3.34 - 4.7 | 5.61 ± 0.46 5.0 - 6.82 | 33 | T2 | 8.42 ± 0.7 7.56 - 9.96 | 12.13 ± 0.89 10.72 - 13.5 |
| 16 | HDE | 6.59 ± 0.67 5.7 - 8 | 8.89 ± 0.71 7.66 - 10.14 | 34 | T3 | 11.97 ± 0.84 10.9 - 14.2 | 17.39 ± 1.18 15.44 - 19.02 |
| 17 | HDAJ | 8.0 ± 0.86 6.62 - 9.2 | 9.97 ± 0.90 8.6 - 11.53 | 35 | T4 | 17.69 ± 1.62 14.8 - 20.54 | 26.06 ± 1.35 23.02 - 28.52 |
| 18 | IAE | 6.74 ± 0.60 5.78 - 7.9 | 8.73 ± 0.48 8.4 - 9.37 | 36 | T5 | 13.34 ± 1.0 12.1 - 15.5 | 19.67 ± 1.31 16.54 - 20.27 |

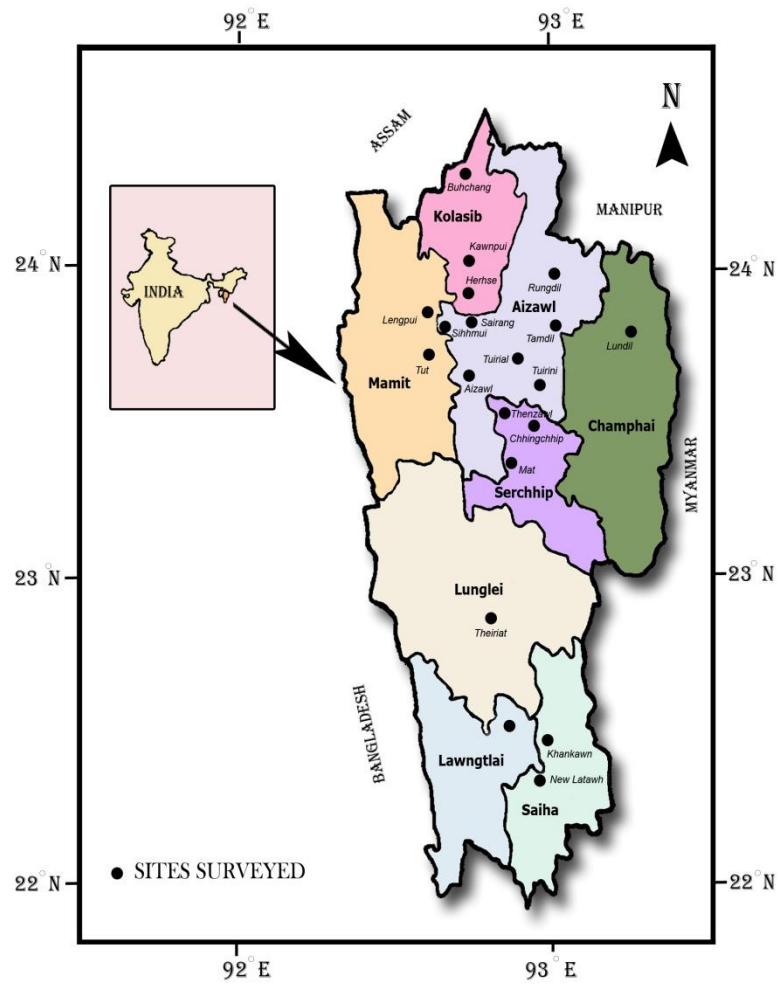


Fig 1.1: Surveyed areas and sites of collections of *Rana alticola*.



- a) Chite River, Aizawl
- b) Rungdíl
- c) Sairang
- d) Sihmui
- e) Tamdíl
- f) Tuirial
- g) Tuirini

Fig. 1.2 (a-g): Study fields at Aizawl District



Fig 1.3 (a-c) : Study fields at Kolasib District



Fig 1.4 : Study field at Champhai District



Fig 1.5 (a & b) : Study fields at Mamit District



Fig 1.6 (a-c) : Study fields at Serchhip District



Fig 1.7 : Study field at Lunglei District



Fig 1.8 : Study field at Lawngtlai District



Fig 1.9 (a-b) : Study fields at Saiha District



Fig 1.10 : Female *Rana alticola*



Fig 1.11(a & b) : Male
Rana alticola

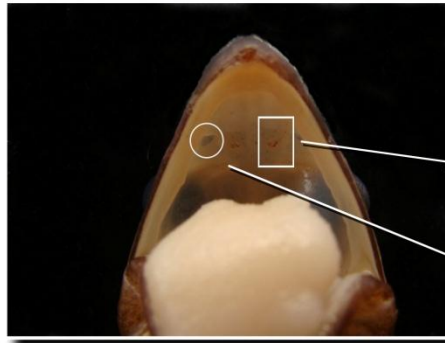


Fig 1.12 : Mouth of *Rana alticola* showing chonae and vomerine teeth



Vomerine teeth



Chonae



Fig 1.13 : Bifid tongue of *Rana alticola*



Fig 1.14: Left hand of *Rana alticola*

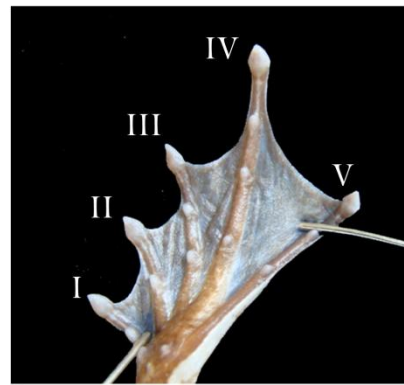


Fig 1.15 : Left foot of *Rana alticola*
Webbing formula: I_{1-1½} II₁₋₂ III₁₋₂ IV₂₋₁ V



Fig 1.16 : Sexual dimorphism in *Rana alticola*



Fig 1.17 : Tadpole showing parotid gland and supracaudal gland

Parotid gland

Supracaudal gland



Ocellus

Fig 1.18 : Tadpole showing Ocellus

Chapter II

Habit, habitat and Breeding behavior
of
Rana alticola

INTRODUCTION

Habitat and resource distributions can influence the movement and aggregation of individuals and thus have important effects on breeding behaviour and ecology. During the non breeding season, most pond-breeding amphibians live in the terrestrial habitat surrounding a pond. In a typical year, adults migrate to the pond during favorable weather conditions to breed. Mating and oviposition usually occur in the pond, and then adults return to their terrestrial habitat. After hatching, the aquatic larvae feed, grow, and develop until metamorphosis, after which they immigrate as juveniles to terrestrial habitats. Amphibian movements around the breeding habitat can be part of normal foraging behavior within an individual's home range (Gibbons and Bennett 1974), or repeated movements to the pond to deposit additional clutches of eggs (Wells 1976; Perrill and Daniels 1983). The terrestrial habitat adjacent to the breeding site provides food and shelter throughout the prolonged breeding season. Amphibians have become increasingly important as indicators of environmental quality because of life history traits that may make them particularly susceptible to environmental contaminants and their important ecological roles in freshwater and terrestrial habitats (Dunson *et al.*, 1992). Most amphibians have complex life cycles, in which different life stages occupy distinct habitat types; thus, susceptibility to specific environmental stresses (e.g.; water borne, soil borne, etc.) varies with life stage. Amphibians also assume very different ecological roles during different parts of their complex life cycle; thus, influences of contaminants on specific life stages would be expected to have broad ecological effects in more than one habitat type. Most frogs and toads, for example, have larval stages that are grazers of living and non-living aquatic plant material, yet on

metamorphosis; they become important predators in terrestrial habitats. Substantial effects of contaminants on the aquatic life stages might not only influence the ecology of the aquatic habitat but also carry over into ecological changes in surrounding terrestrial habitats via decreased recruitment of predatory juvenile.

Among anurans, two basic reproductive patterns are evident. Most tropical and subtropical species are capable of reproduction throughout the year; rainfall seems to be the primary extrinsic factor controlling the timing of reproductive activity. In most temperate species, reproductive activity is cyclic and dependent on a combination of temperature and rainfall. In seasonally dry tropics, anuran reproductive activity is closely associated with the rainy season. The greatest diversity of reproductive modes is seen among anurans in the tropics. Temporal patterns of anuran reproduction vary widely, but can be roughly divided into two broad types: explosive breeding and prolonged breeding (Wells 1977a).

Practically all anurans have external fertilization. However, within the constraints of external fertilization, anurans have a great diversity of reproductive modes. The most common and phylogenetically widespread site of oviposition is in free water; standing or flowing, permanent or temporary. Those frogs that oviposit in ephemeral aquatic sites are dependent upon heavy rainfall; those that utilize streams usually breed at times of little rainfall, when the water level is low and the current is slow. Thus, even though temperature and moisture may be sufficient throughout the year, unpredictability of oviposition sites probably restricts reproduction activity. In most anurans, fertilization occurs externally at the time of oviposition and the pair is in amplexus and there is some evidence for various kinds of tactile signals between the sexes. Once the female has selected an oviposition site, peristaltic abdominal

contractions in the female accompany ovulation or movement of eggs down the oviduct and may signal the male that oviposition is about to take place. Oviposition and fertilization are accomplished by synchronized movements by both partners. Mode of reproduction is a combination of oviposition site and type of egg development.

During the breeding season male frogs vocalize to attract females. There is considerable variation among anurans in terms of the diversity and complexity of their vocal repertoires (Littlejohn 1977). While a large proportion of species have a male advertisement call, a minority has, in addition, distinct aggressive or territorial calls and encounter calls. The loud, explosive distress calls given in response to acute disturbance or grasping by a potential predator are produced by either sex or sometimes even newly metamorphosed young (Sazima, 1975), and are acoustically dissimilar to the advertisement calls. In producing sound, the anuran larynx is the transducer that converts muscular activity into acoustic energy through the manipulation of air flow by the force-pump mechanism. In most species there is great potential for competition among conspecific males because operational sex ratios are strongly male- biased (Arak, 1983). This competition is conspicuous in many prolonged breeding species that use physical combat to defend territories, and complex vocal repertoires to influence the outcome of disputes (Wells, 1977a; Given, 1987, 1988a). Combat is a feature of the reproductive behaviour of many amphibians; typically males fight for females or for a resource that is prerequisite for attracting females, such as a call site, a territory, or an oviposition site.

When both the male and the female are ready to mate, the male usually grasps the female so that he is dorsal to her. This embrace, amplexus, is inguinal in

the primitive frogs, including all archaeobatrachians, myobatrachids, and some telmatobiine leptodactylids (Lynch, 1973), and Sooglossids (Nussbaum, 1980). With the male's forelimbs grasping the female around the waist, the vents are not juxtaposed; presumably this method of amplexus is not as efficient for ensuring fertilization of eggs as is the more forward position, axillary amplexus, which places the vent closer together (Rabb, 1973). Most neobatrachians have axillary amplexus. Axillary amplexus normally involves the male grasping the female in the axilla; in those species having nuptial excrescences, the pads or spines are pressed tightly into the axilla.

In addition to the reproductive organs and their associated tracts, external sexual differences exist in most amphibians, including size, glanular development, skin texture, dermal ornamentation, vocal sacs, and colouration. Some differences persist throughout adult life, but others develop in response to gonadotropic hormones and therefore are present only during the active reproductive cycle. Some structures are used in courtship and others, for holding the pair in an embrace during mating or oviposition. Nuptial pads enhance a male's grip on the female during amplexus, enabling him to resist attacks by rivals more effectively. Savage (1935) suggested that nuptial pads are analogous to male weapons, because of their role in competition for females, and suggested that they are evolved most highly in those species in which male-male competition is more severe.

REVIEW OF LITERATURE

Despite the high diversity of anuran communities in the tropics, basic information on habitat use and reproductive patterns are available for relatively few tropical anuran assemblages (Aichinger 1987, Berry 1964, Crump 1974, Donnelly & Guyer 1994, Gascon 1991, Heyer 1973, Inger 1969, Trennery 1991). Wetlands provide critical habitat for a wide variety of amphibian species. However, many amphibian species also require terrestrial habitat adjacent to wetlands to complete their life cycle (Semlitsch, 1998). Temporal patterns of anuran reproduction vary widely, but can be roughly divided into two broad types: explosive breeding and prolonged breeding (Wells 1977a). In many species, the length of the breeding period is limited by climate, or the seasonal availability of breeding sites, such as ephemeral pools. Temperate species are more likely to breed explosively—an entire population breeding over a span of a few days or weeks. By contrast, many tropical anurans breed in every month of the year, although most demonstrate some seasonality as well. Some tropical species restrict their breeding activity to either the wet or the dry season, but this does not tend to limit their breeding season to the same degree that cold limits breeding in the temperate zone (Wells 1977a). The diversity of life history strategies found in anurans is remarkable. Restricted, in most cases, by external fertilization, and by the need to find moist habitats for their eggs, anurans have nevertheless evolved myriad ways to thrive. Indeed, anuran reproductive modes are so diverse that there is still no single, universally cited source that encompasses all recognized modes. Furthermore, it appears that trends away from the primitive mode of eggs and tadpoles in ponds do not reflect one or

two evolutions of increasing specialization, but many independently derived reproductive modes in distinct phylogenetic lineages (Duellman and Trueb 1986).

Breeding: Anuran breeding biology is influenced by climatic factors like temperature and rainfall or a combination of these, both for tropical and temperate species (Duellman and Trueb 1986; Beebee 1995a). Literature on breeding activity for various species show that breeding activity is govern by either a single factor-temperature (Briggs, 1987; Fukuyama & Kusano, 1992), rainfall (Ritke *et al.*, 1992; Donnelly & Guyer, 1994), moisture (Doreas & Foltz, 1991; Moreira & Barreto, 1997), wind (Robertson, 1986) or a combination (Lizana *et al.*, 1994; Moreira & Barreto, 1997). Diaz-Paez and Ortiz (2001) stated that reproductive patterns are correlated with prevailing climatic conditions. Species that inhabit temperate zones, exhibit a determined gonadal annual cycle, with alternation of activity and resting periods, where the reproductive standards reflect the annual climatic cycles (Jorgensen, 1981, 1984; Jorgensen *et al.*, 1978, Jorgensen *et al.*, 1986; Rastogi *et al.*, 1976, Rastogi *et al.*, 1983a). Species that inhabit tropical areas, with a constantly warm and humid climate, exhibit continuous or potentially continuous cycles (Church, 1960; Inger & Greenberg, 1963; Jorgensen *et al.*, 1986; Rastogi *et al.*, 1976). However, environmental variables such as humidity, temperature and photoperiod may determine anuran breeding period (Navas, 1996; Navas and Bevier, 2001; Hatano *et al.*, 2002). The annual reproductive pattern of amphibian has been studied extensively from the temperate and subtropical regions, and the physiological basis for their cyclic reproductive behaviour has been dealt with (Inger and Bacon Jr., 1968). Furthermore, other factors may interfere with reproduction, such as temperature intrinsic (morphophysiologic) components, water temperature,

predation pressure, and competition for food. The reproductive activity of anurans living in seasonal environments is generally associated with the rainy periods, both in tropical climates (Hoogmoed and Gorzula, 1979, Aichinger, 1987) and in temperate ones (Banks and Beebee, 1986; Caldwell, 1987). It is not uncommon, however, especially in tropical areas, to find anurans reproducing year-round even in seasonal environments (Aichinger, 1987; Barreto and Moriera, 1996), provided favorable sites for reproduction are available throughout the year. Rain is the primary extrinsic factor affecting the time of the breeding activity for most tropical and subtropical anuran species (Haddad and Sazima, 1992; Duellman and Trueb, 1994). Anurans from tropical and subtropical regions frequently depends on rain for their breeding activity (Duellman and Trueb, 1994) although rainfall is associated with the breeding in some species; it seems to have less influence once the breeding starts (Okuno, 1985). Periods of rain or drought affect the presence of suitable places to spawn, and thus are a limiting factor for the reproduction of water-dependent species (Ryan, 1985; Díaz-Paniagua, 1990). The unpredictability of reproduction sites can be compensated for by some flexibility in the breeding season (Díaz-Paniagua, 1990).

Gonadal sex differentiation among amphibians has been described in *Rana sylvatica*, *Rana temporaria*, *Rana cantabrigensis*, *Rana catesbeiana*, *Rana ridibunda*, *Rana nigromaculata*, *Rana ornativentris*, *Rana esculentia*, *Bufo japonicas* and *Rana curtipes* (Witschi, 1914, 1929; Cheng, 1932; Padoa, 1942; Iwasawa, 1969; Hsu and Liang, 1970; Shirane, 1986; Ogielksa and Wagner, 1990; Gramapurohit *et al.*, 2000). Among anuran amphibians cyclic ovarian changes have been reported in *Xenopus laevis* (Dumont 1972) ; *Bufo bufo* (Jorgensen *et al.*, 1979);

Rana esculenta (Rastogi *et al.*, 1983b); *Rana (Euphlyctis) cyanophlyctis* (Pancharatna and Saidapur 1985); *Pachymedusa dacnicolor* (Iela *et al.*, 1986); *Bufo (Duttaphrynus) melanostictus* (Kanamadi *et al.*, 1989); *Rana perezii* (Delgado *et al.*, 1990); *Polypedates maculatus* (Kanamadi and Jirankali, 1991); and *Hyla pulchella andina* (Montero and Pisano, 1991). These studies reveal that amphibians have diverse reproductive cycles and, as a result, various reproductive outputs (Saidapur 1989). Temperate zone female anurans typically have annual ovarian cycles that are seasonally correlated (Jorgensen *et al.*, 1979; Rastogi *et al.*, 1983b). In contrast, tropical anurans have diverse patterns of ovarian follicular development even among sympatric species (Saidapur 1989). There have also been numerous studies on the testicular cycle of many anuran species including *Rana iberica* (Crespo and Cei, 1971, 1973); *R. temporaria* (Koskela *et al.*, 1979; Arrayago and Bea, 1986); *R. catesbeiana* (Swingle, 1921); *R. esculenta* (Lofts, 1964); *R. nigromaculata* (Satoh, 1971); *R. perezii* (Delgado *et al.*, 1989a); *Alytes obstetricians* (Crespo, 1982); and *Discoglossus pictus* (Champy, 1913).

Reproductive mode: Crump (1974) defined reproductive mode simply as the combination of deposition site and type of development. Reproductive mode is defined by Salthe and Duellman (1973), and used by Duellman and Trueb (1986) as a “combination of ovipositional and developmental factors, including oviposition site, ovum and clutch characteristics, rate and duration of development, stage and size of hatching, and 15 type of parental care, if any.” Brown and Alcalá (1983) explicitly add larval nourishment to this list. Crump (1974) proposed 10 reproductive modes to describe the anurans of the upper Amazon basin in 18 eastern Ecuador. Salthe and Duellman (1973) listed nine to represent all of Anura, but they excluded

viviparity from consideration. Lamotte and Lescure (1977) broke anuran reproduction into six primary categories, and 18 specific modes. Brown and Alcala (1983), in their review of reproductive modes in Philippine anurans, identified the same two general modes used by Jameson (1957) and Salthe and Mecham (1974), but they recognized 18 specific modes. Duellman and Trueb (1986) proposed three general modes (eggs aquatic, eggs terrestrial or arboreal, and eggs retained in oviducts), and divided these into 29 specific modes. Jørgensen (1992) approached the problem qualitatively, and declined to fully separate reproductive mode from reproductive pattern, finding that climatic conditions, latitude, altitude, seasonality, the length of the breeding season and courtship are all inextricably tied to reproductive mode. It is commonly presumed that the primitive reproductive mode for anurans is by means of large numbers of relatively small aquatic eggs deposited into lentic water, in which the tadpoles also develop (Duellman and Trueb 1986, Jørgensen 1992). Duellman and Trueb (1994) describe the reproductive mode of a species as composed by different factors such as breeding site, egg and clutch characteristics, rates of egg development, size and conditions of the larva at the moment of birth, among others. Neotropical anurans display the greatest diversity of reproductive modes among the amphibians, with more than 30 different modes (Haddad and Sawaya, 2000).

The diversity of life history strategies found in anurans is remarkable. Restricted, in most cases, by external fertilization, and by the need to find moist habitats for their eggs, anurans have nevertheless evolved myriad ways to thrive. Indeed, anuran reproductive modes are so diverse that there is still no single, universally cited source that encompasses all recognized modes. Furthermore, it

appears that trends away from the primitive mode of eggs and tadpoles in ponds do not reflect one or two evolutions of increasing specialization, but many independently derived reproductive modes in distinct phylogenetic lineages (Duellman and Trueb 1986).

Vocalization: Acoustic communication constitutes an important and conspicuous part of the breeding biology of most anurans. It is involved in the establishment and maintenance of territories by the males, in facilitating the attraction of conspecific females to the males, in courtship, and in the identification of sex and reproductive state (Littlejohn 1977). Calling is an energetically expensive activity for males (Pough et al. 1992). There are a number of ways in which males can alter their calls in response to competition from rivals, such as; increasing call intensity, increasing call duration, increasing note repetition rate, adding extra notes to the call, and adding new notes to the call (Wells 1977c; Wells 1988). Early reviews of anuran vocalizations (Bogert, 1960; Blair, 1963; Paillette, 1971) were concerned primarily with the evolutionary significance of vocalization at population and species levels; Straughan (1973) provided an overview of evolution of vocalization in anurans. Gans (1973) and Schmidt (1973) provided insights into the mechanisms and control of vocalizations. Syntheses by Wells (1977a, 1977b) and Littlejohn (1977) emphasized the evolutionary and ecological interactions at the individual level. Workers like Roy and Elepfandt 1993; Roy 1994; Roy et al. 1995; Ryan 1985, 1986; Schneider and Sinsch 1992; Sinsch and Schneider 1996 has demonstrated that anuran mating calls constitute an important character for species identification, more distinctive than morphological characters. Roy and Elepfandt (1993) recorded three different advertisement calls from frogs, all of which were

identified morphologically as *Rana limnocharis*, currently known as *Fejarvarya limnocharis*. Similar results have been reported by Sharma (1996). Anuran reproduction patterns range from explosive (short-duration) to prolonged breeding (Wells 1977a). In prolonged breeders, males attract females with advertisement calls (Gerhardt 1994) and may defend oviposition sites (Wells 1977b; Howard 1978b), therefore female choice (and males' ability to attract females) is a determinant of male mating success (Ryan 1980, 1983; Arak 1983; Sullivan 1985; Robertson 1986; Marquez and Tejedo-Madueno 1990). This leads to vocal competition or the defence on individual territories.

Reviews dealing with various aspects of vocalization in ranids have been reported. Matsui *et al.* (1993) described acoustic characteristics of three species of the Genus *Amolops* (Ranidae) namely *Amolops chunganensis* from western China, *A. larutensis* from Peninsular Malaysia and *A. jerboa* from Borneo. Given (1999) demonstrated the advertisement calls of male carpenter frogs (*Rana virgatipes*). Wycherley *et al.*, (2001) developed new computer analytical techniques that aid bioacoustic separation of Norfolk pool frog, *Rana lessonae* populations. Bee (2002) studied on the socially mediated pitch alteration by territorial male bullfrogs, *Rana catesbeiana*. Wycherley *et al.*, (2003) applied male advertisement call characteristics for the identification of introduced water frogs in Britain. Grosjean and Dubois (2005) described in details the advertisement calls of six species of the genus *Chaparana* (subgenus *Paa*), of which two of them (*Chaparana minica* and *Chaparana vicina*) were for the first time. A Synopsis of Bioacoustic Studies of Anuran Amphibians of Borneo was described by Sukumaran and Das (2006) where

they reported that the calls of 101 species of anuran amphibians (65.6%) are known from Borneo.

In India, Kanamadi *et al.*, (1994) presented the advertisement calls of *Rana tigrina* and *Tomopterna breviceps* that occur as sympatric species at Dharwad. Roy (1997) made a comparison on the male advertisement call with female reciprocal call in *Limnonectes (Fejervarya) limnocharis*, *Euphlyctis cyanophlyctis* and *Polypedates leucomystax*. Roy *et al.*, (1998) provided the first detailed account, describing absolute measurements, biometric ratios and call pattern in terms of temporal and spectral characters of 10 Indian amphibian species namely – *Amolops formosus*, *Bufo (Duttaphrynus) melanostictus*, *Euphlyctis cyanophlyctis*, *Hoplobatrachus tigerina*, *Hyla annectens*, *Limnonectes (Fejervarya) limnocharis*, *Limnonectes (Fejervarya) khasiana*, *Polypedates leucomystax*, *Polypedates maculatus* and *Rana alticola*. In northeast India, analysis of anuran advertisement call limited to *Microhyla heymonsi* from Assam (Grosselet *et al.*, 2004).

Courtship and Mating: The term courtship is used to refer to the interaction between males and females leading to pair formation and mating. The courtship may be simple in that the male may call until a female contact him; then he clasps the females (Wells, 1977a). An important effect of an increased tendency among researchers to study the behavior of individually marked animals has been to show that different individuals may use very different behavior patterns during competition for a limited resource such as mates. These different patterns are called alternative mating strategies (Arak 1984). Amphibians have proved to be a rich source of alternative mating strategies, and provide some of the best-studied examples. In anurans one can find alternative non-calling behavioural mating tactics,

such as males actively moving around the breeding area seeking females, eg, *Bufo bufo* (Davies and Halliday 1979), *Rana sylvatica* (Howard 1980; Woolbright *et al.*, 1990) and *Bufo calamita* (Arak 1988b; Tejedo 1992). Occasionally males fight for the possession of female already in amplexus, and engage in protracted struggles, eg *Bufo bufo* (Davies and Halliday 1979), *B.americanus* (Howard 1988), *B.gutturalis* (Telford and van Sickle 1989) and *Rana sylvatica* (Howard and Kluge 1985). Both these tactics are typical of explosive breeders and their occurrence is density-dependent (Woolbright *et al.*, 1990). In some anurans, especially in those with prolonged breeding seasons, alternative strategies take the form of caller-satellite associations. It is expected that when mating competition is severe, disadvantaged males will use alternative mating tactics to enhance their mating probabilities (Perrill 1984; Anderson 1994; Lucas and Howard 1995). For example, one of the best known alternative behaviors of chorusing anurans is the “satellite tactic” (silent males around callers), which has been modeled as a dynamic game (Waltz 1982; Lucas *et al.*, 1996). The satellite behavior or “sexual parasitism” has been reported for various species of *Bufo*, *Hyla*, *Pseudacris*, *Rana*, and *Gastrophryne* (Axtell, 1958; Brown and Pierce, 1967; Wells, 1977c, 1978; Howard, 1978a; Perrill *et al.*, 1978; Fellers, 1979a, b; Miyamoto and Cane, 1980; Godwin and Roble, 1983). In the species of *Hyla* and *Pseudacris nigrita*, there is no size difference between calling and satellite males. Furthermore, in *H. cinerea*, *H. chrysoscelis*, and *P. nigrita*, individual males changed strategies from calling to satellite behavior or from satellite to calling behavior on different nights or on the same night (Perrill *et al.*, 1978; Godwin and Roble, 1983). In most species there is great potential for competition among conspecific males because operational sex ratios are strongly male- biased (Arak,

1983). This competition is conspicuous in many prolonged breeding species that use physical combat to defend territories, and complex vocal repertoires to influence the outcome of disputes (Wells, 1977b; Given, 1987, 1988a, b). Combat is a feature of the reproductive behaviour of many amphibians; typically males fight for females or for a resource that is prerequisite for attracting females, such as a call site, a territory, or an oviposition site. Low energetic cost tactics are expected to be adopted by disadvantaged, i.e., youngest or smallest, males (Waltz 1982; Krupa 1989; Lucas and Howard 1995). Among anurans, males of *Acris crepitans blanchardi* (Wagner 1989) and *Rana virgatipes* (Given 1988a) engage in wrestling fights over calling sites; in these bouts larger males have a clear advantage. In *Oloolygon rubra*, a lekking species, unpaired males attack amplexant males that females have actively chosen and, if they are larger than amplexant males, can displace them (Bourne 1992). Davies and Halliday (1979) found that nearly 40% of mating males obtained their partners by displacing a rival from amplexus, in fights that last for an average of seven hours (Davies and Halliday 1977). Bourne (1992) suggested that it is the larger arms of certain males, rather than their overall body size, that enables them to resist the attacks of rivals attempting to displace them from amplexus.

Amplexus: Reports of multiple male frogs in amplexus with a single female occur sporadically in many frog families (Byrne & Roberts 2004). In some cases, these sorts of associations lead to polyandrous fertilisation of eggs from a single clutch (D'orgeix and Turner 1995; Roberts *et al.*, 1999) and polyandry is reasonably suspected in others (Jennions and Passmore 1993) many multiple male, single female associations in frogs may actually involve a real risk of sperm competition. Prolonged amplexus in anuran may be a strategy for monopolizing a female,

especially if paring takes place before ovulation (Wells, 1977a). Amplexus takes place at the breeding site and may last for 12 days in *Rana temporaria* (Geisselmann *et al.*, 1971) or 14 days in *Bufo bufo* (Heusser, 1963). Observations of unmated males attempting to dislodge amplexing males indicate that most mated males maintain amplexus and therefore are affectively monopolizing the mated female.

Oviposition: In most anurans, fertilization occurs externally at the time of oviposition and the pair is in amplexus and there is some evidence for various kinds of tactile signals between the sexes. Oviposition sites act as patches, each of which contain various degrees of resource and risk (Resetarits Jr. 1996). Several studies have revealed oviposition site preference for a wetter, or potentially wetter, environment in amphibians (treefrogs: Crump 1991; salamanders: Figiel and Semlitsch 1995; ranid frogs: Spieler and Linsenmair 1997). Oviposition sites are a critical parameter of reproductive success in any species that does not move it's young immediately after laying.

Clutch: Clutch size is an important demographic trait of amphibians that is frequently used in descriptive ecological studies (Woodward, 1982), biogeographic comparisons (Karraker *et al.*, 2006), studies of population dynamics (Berven, 1990) and population modelling (Vonesh and De la Cruz, 2002). In *Rana tigerina* the eggs are laid in a clutch in shallow water and the clutch size ranges from 4660 to 6460. There is no correlation between the eggs and the female size (Dutta and Mohanty-Hejmadi, 1976). *Rana cyanophlyctis* clutch ranges from 300 to 500 (Mohanty-Hejmadi and Dutta, 1978). The egg clutches of *C. machalilla* ranges from 8-21 eggs (Del Pino *et al.*, 2004). Clutches of *Scinax trapicheiroi* consist of a circular, laminar gel mass containing the eggs, which are laid on the water surface, sometimes

adhered to the superficial or marginal vegetation and the clutch ranges from 115-609 eggs (Rico *et al.*, 2004).

Multiple clutches has been shown in several anuran families like Hylidae; *Hyla chrysoscelis* (Godwin and Roble 1983; Ritke *et al.*, 1992), *Hyla cinerea*, *H. regilla* and *H. gratoisa* (Perrill and Daniel 1983), *Pachymedusa dacnicolor* (Iela *et al.*, 1986), Ranidae; *Rana clamitans* (Wells 1976), *Rana catesbeiana* (Emlen 1977; Howard 1978b), *Rana esculenta* (Rastogi *et al.*, 1983a). Hyperolidae: *Hyperolius viridiflavus* (Richards 1977), *Hyperolius marmoratus* (Telford and Dyson 1990), Bufonidae; *Bufo calamita* (Banks and Beebee 1986), Discoglossidae; *Discoglossus pictus* (Knoepffler 1962), *Bombina variegata* and *B. bombina* (Obert 1977; Kapfberger 1984); *Alytes muletensis* (Bush 1993); Dendrobatidae; *Dendrobates granuliferus* (Crump 1972). Myobatrachidae: *Crinia signifera* (Lemckert and Shine 1993).

Sexual dimorphism: Males and females of a same species frequently show great differences in life history, morphology and behavior. This sexual dimorphism has been explained as a consequence of inter-sexual differences in direction and intensity of sexual selection and natural selection (Shine, 1989; Anderson, 1994). In most species, sexual selection is especially strong in males, and promotes the evolution of morphologies and behaviours that increase their chances of mating (Anderson, 1994). On the other hand, natural selection can favour different adaptations in males and females that reduce competition between sexes of a same species for habitat and/or prey (Shine, 1989). In anurans a conspicuous sexual dimorphism is the presence of spines, tusks and the call behaviour of males (Shine, 1979; Duellman and Trueb, 1994). However, the more intriguing aspect of sexual

dimorphism in anurans is the difference in body size (Shine, 1979, 1989; Woolbright, 1983; Monnet and Cherry, 2002). Female exhibits larger body size than males in 90% of anurans species (Shine, 1979). Several authors point sexual selection as the main cause of this sexual size dimorphism (SSD) (Shine, 1979; Woolbrigh, 1983; Halliday and Tejedo, 1995) but others claim that demographics also can play an important role (Arak, 1988; Monnet and Cherry, 2002; Gramapurohit *et al.*, 2004). Many studies have demonstrated that female size correlates positively with the number of eggs laid or ovarian eggs produced (Martins, 1988, Ryser, 1988, Praderio and Robinson, 1990).

Secondary characters: There is considerable variation among species in the form of these secondary sexual characters; some species have glandular pads, others a single thorn-like spine, others hundred of very small spines (Smith 1951; Noble 1954; Tyler 1976; Duellman and Trueb 1986). In some species that breed in fast flowing water, males have similar structures on their chests (Smith 1951; Duellman and Trueb 1986). Thomas *et al.*, (1993) identified a category of sexually dimorphic skin glands, present in the nuptial pads of many taxa, and, in some species, elsewhere on the body, that may secrete adhesive secretions that enhance a male's ability to hold onto a female. Many male anurans possess nuptial pads on the forelimbs that first appear at sexual maturity (Jorgensen 1992) and are developed most fully in the breeding season.

In India the breeding behaviour of ranids was observed by few workers, *Rana (Hoplobatrachus) tigerina* (McCann, 1932; Dutta and Mohanty-Hejmadi, 1976), *Rana (Euphlyctis) cyanophlyctis* (Mohanty-Hejmadi and Dutta, 1978; Mallick, 1988).

MATERIALS AND METHODS

The present study is concerned with the breeding behavior, habit and habitat of *Rana alticola* in Mizoram. For the purpose of this study, three study sites were selected in Mizoram (Fig 2.1). The three study sites are Sairang River (Fig 2.2, 2.3), Herhse stream (Fig 2.5, 2.6) and Tamdil Lake (Fig 2.8, 2.9) designated as study site I, II and III respectively. Habitat of the three study sites were conducted to record the topography, vegetation, and ecological factors.

Study site I: The study site I is at Sairang River (23^o48'N and 92^o37'E), which is a part of Tlawng river, the longest river in Mizoram and is considered the most important river in the state.

Study site II: The study site II is at Herhse stream (23^o58'N and 92^o41'E), which is one of the tributaries of Tuitun river, one of the major rivers in Mizoram.

Study site III: The study site III is Tamdil Lake (23^o44'N and 92^o57'E), which has been identified as wetland under National wetland conservation programme 2006 – 2007 by the Government of India.

To determine the breeding period, observations were carried out both in the natural and laboratory condition. In the natural condition, observation was carried out with the help of photography with canon camera: EOS digital rebel XTi/EOS 400D digital. Audio Encounter Surveys (AES) and Acoustic Encounter Surveys (AES) were used to identify the locations of the calling males. Individuals were captured by hand or net. Presence of gravid female and eggs at the study sites gives evidence that it is the breeding season. Headlamps, torchlight and bamboo torch (local tradition) were used to locate eggs and amplexing pairs. Field sampling was

carried out at weekly intervals; each sampling session spanning over two to three continuous days.

Amplectant pairs were collected so that clutches could be obtained in the laboratory for determining the number of eggs per clutch (clutch size). Following capture, animals were transported to the laboratory and snout-vent lengths (SVL) were determined to the nearest 0.02 mm with a Mitutoyo dial caliper. When the female oviposits, the time of oviposition and the time taken by the female to oviposit were recorded. Then the numbers of eggs were counted to know the clutch size. Ecological factors like temperature of air and water, relative humidity, rainfall and pH were recorded at a weekly interval from the year 2005 to 2007. Temperature was recorded with the help of a thermometer, relative humidity with the help of a hygrometer and pH with the help of a pH pen. Rainfall data was obtained from The Department of Agriculture and Minor Irrigation, Mizoram.

The vegetations present in and around the breeding sites were also recorded and identified. Collection and preservation of plant specimens were done following the method given by Jain and Rao (1976). Identification of the collected specimens was done by comparing with herbarium specimens in the Department of Botany, North Eastern Hill University, Shillong.

The calls were recorded with the help of a digital voice recorder model Samsung SVR 380 and transferred to PC and analyzed with the software 'sound ruler Acoustic Analysis Version 0.9.6.0. Sound Ruler is an analysis, graphing and teaching tool for bioacoustics. The sampling rate used to convert the signals to digital format was 8 KHz with 16-bit precision. The oscillogram was prepared and analyzed with the help of a software tool "SoundRuler Version 0.9.6.0 (acoustic

analysis)”. The notes are composed of groups of pulses. Notes are measured from the beginning of the first pulse to the end of the last pulse; intervals between two subsequent notes are measured from the end of the last pulse of the first note to the beginning of the first pulse of the following note; note repetition rate is the number of notes per second; pulse repetition rate is the number of pulses per second.

The breeding activity was also studied based on the observations recorded at the breeding site. During the present study, the structure of the testis and ovary of the adult frog was also studied. For this purpose, adult male and female were collected from the study site and brought to the laboratory. The frogs were euthanized in MS-222 and ovaries and testis were excised and immediately fixed in Bouin’s fixative. This fixation is done right after dissection so as to avoid post-mortem changes. The tissues were kept in Bouin’s fixative for 24 hours and then dehydrated through several grades of alcohol. After dehydration, the tissues were cleared in xylene and then embedded in paraffin and allowed to solidify. They were then serially sectioned at 7-8 μ thickness and stained with haematoxylin and counter-stained with eosin. The sections were then observed under the microscope Leitz Ortholux 2. Photographs of the histological sections were taken with the help of Leitz Ortholux 2 microscope fitted with photographic attachments.

RESULTS

The breeding behavior of *Rana alticola* was observed from the three study sites namely Sairang River (Study site I), Herhse stream (Study site II) and Tamdil Lake (Study site III) located within Mizoram (Fig 2.1). The habitats of the breeding sites are as follows:

Study site I: The study site I is at Sairang which is an important town situated at Aizawl district. It is about 25 km from Aizawl city. It lies between 23°48'N and 92°37'E at an altitude of 50-80 m asl. The Tlawng River, which is the longest river and also the most important river in Mizoram pass through this town. The length of this river is 185.15 kms. It flows south to north and joins the Barak in Assam's Cachar district. Sairang is situated about 22 kms from Aizawl, the capital of Mizoram (Fig 2.2 and 2.3). The main vegetations around this area are *Osbeckia sp.*, *Pteris sp.*, *Centella asiatica*, *Ageratum conyzoides*, *Chromolaena odorata*, *Colocasia sp.*, *Mikania micrantha*, *Mimosa pudica*, *Erythrina variegata*, *Michelia champaca*, *Schima wallichii*, *Stereospermum sp.*, *Lagerstroemia speciosa*, *Ageratum conyzoides*, *Piper*, *Alternanthera*, *Tectona grandis*, etc (Fig 2.4)

Study site II: The study site II is at Herhse stream (23°58'N and 92°41'E), which is one of the tributaries of Tuitun river, one of the major rivers in Mizoram. This study site is a stream with thick forest cover. The water flows from north to south direction. The elevation is about 308 m asl and is situated about 60 kms from Aizawl (Fig 2.5 and 2.6). The area is dominated by shrubby vegetation like *Ageratum conyzoides*, *Bidens biternata*, *Crassocephalum crepidioides*, *Osbeckia crinata*, *Eupatorium riparium*, *Colocasia sp.*, *Hedychium sp.*, *Pteris sp.*, *Mussaenda glabra*, *Spilanthes acmella*, *Thysanolaena maxima*, *Chromolaena odorata*, etc.,

bamboos like *Dendrocalamus* sps., *Neohouzeua dulloa*, *Bambusa arundunacea* etc. and trees like *Careya arborea*, *Shorea robusta*, *Tectona grandis*, *Duabanga grandiflora*, *Mesua ferrea*, *Michelia champaca*, *Schima wallichii* (Fig 2.7).

Study site III: The study site III is Tamdil Lake (23⁰44'N and 92⁰31'E), the elevation of this study site is 760 m asl. Tamdil Lake has been identified as wetland under National wetland conservation programme 2006 – 2007 by the Government of India. The Tamdil Lake is situated 110 kms from Aizawl (Fig 2.8 and 2.9). The lake is surrounded by tropical evergreen and moist deciduous forest with species like *Schima wallichii*, *Chukrasia tabularis*, *Albizzia* sp., *Artocarpus* sp., *Morus* sp. The aquatic fauna consists of fish and the surrounding forests abound in bear, deer, wild pig and other wild animals. The main vegetations found in and around this field are, *Dendrocalamus* sp., *Eichornia crassipes*, *Spilanthes acmella*, *Mikania*, *Polygonum chinensis*, *Commelina bengalensis*, *Mimosa pudica*, *Impatiens*, *Polygonum hydropiper*, *Drymaria cordata*, and species belonging to the family *Verbenaceae*, *Poaceae*, *Melastomaceae* and *Cucurbitaceae*. Trees like *Tetrameles nudiflora*, *Gmelina arborea*, *Artocarpus lakoocha*, *Artocarpus heterophyllus*, *Musa paradisiaca*, *Spilanthes acmella*, *Carica papaya*, etc. (Fig 2.10).

Breeding season: Observations in the field during the study period indicate that the breeding season of *Rana alticola* starts from late June which coincides with rainy season and continue till early October where the level of the water is comparatively high (Fig 2.11a, 2.12a and 2.13a) as compared to the non-breeding season where the level of the water is low (Fig 2.11b, 2.12b and 2.13b).

Emergence/ pre breeding: During the study periods from 2005 to 2007, in all the three study sites, *Rana alticola* adults start to emerge from late June (Fig 2.14

and 2.15) where heavy shower of rain was recorded at 673.1 mm at study site II in the year 2006 and the lowest rainfall recorded was 113.2 mm at study site III in the year 2005 (Table 2.1; Fig 2.29, 2.30 & 2.31). The lowest relative humidity recorded was 24% at study site I in the year 2005 and highest was 98 % at study sites I and II in the year 2005 (Table 2.2; Fig 2.32, 2.33 & 2.34). Also the lowest air temperature recorded was 16°C at study site I in the year 2005 and the highest recorded was 34°C at study site II in the year 2006 (Table 2.3; Fig 2.35, 2.36 & 2.37). Lowest water temperature recorded was 15°C at study site I in the year 2005 and the highest recorded was 26°C at study site I in the year 2007 (Table 2.4; Fig 2.38, 2.39 & 2.40), and pH ranged from 6.5 to 7.1 (Table 2.5; Fig 2.41, 2.42 & 2.43). It was observed that the male emerged first and reaches the breeding site first, and then they start the advertisement calls (Fig 2.16) which attract the receptive female towards the breeding site (Fig. 2.17). Breeding activities starts with the emergence from late June and continue till early October. During October, in all the three study sites from the year 2005 to 2007, rainfall ranged from 67 mm to 269.3 mm (Table 2.1; Fig 2.29, 2.30 & 2.31). The relative humidity ranged from 39% to 97% (Table 2.2; Fig 2.32, 2.33 & 2.34). The air temperature recorded was 17°C to 35°C (Table 2.3; Fig 2.35, 2.36 & 2.37). The water temperature ranged from 13°C to 21°C (Table 2.4; Fig 2.38, 2.39 & 2.40) and pH ranged from 6.5 to 7 (Table 2.5; Fig 2.41, 2.42 & 2.43).

Breeding / Courtship and Mating: Following the emergence, the adult males start the advertisement calls which are in chorus.

Vocalization: During the breeding season, the males of *Rana alticola* started calling from 1400 hours till 2100 hours from the month of June following the emergence. The adult males were found croaking within the vegetations present in

and around the water bodies. Since *Rana alticola* has an internal vocal sac, the only way to distinguish between the male and female is from the size where the female is always bigger than the male. The call sounds like the chirping of bird with a ‘chirp’ sound. Both male and female adults also give a distress call when in captivity which differ from the advertisement call.

Advertisement call: The call consisted of four notes and the call duration lasted for one second (Fig 2.18a). Each note consists of two to three pulses and each note lasts for 0.1 second (Fig 2.18b). The dominant frequency spread over a frequency band of 1636.52 – 2497.85 Hz.

Distress call: The call consisted of two notes and the call duration lasted for two seconds (Fig 2.19a). Each note consists of six pulses and each note lasts for 0.3 second (Fig 2.19b). The dominant frequency spread over a frequency band of 1335.06 – 1421.19 Hz.

The male arrive first at the breeding site and start the advertisement call which attract the female to come towards the breeding site for mating. Generally the individuals were observed associated with plants whose leaves are positioned on the water surface like that of *Eichornia crassipes* and *Mikania*. On such leaves, males were found calling both nocturnally and diurnally (Fig 2.15 and 2.16). The advertisement call is the major factor in the courtship of *Rana alticola* which initiate the female to approach the calling male, the male will grasp the female by the armpit and amplexus will start. Amplexus is axillary where the male grasps the female at the axilla (Fig 2.20). The male arches his back dorsally, brings his cloaca into juxtaposition with the female, and ejaculates sperms as eggs are deposited by the

female. The male moves its body in an up down movement while in amplexus with the female.

It was observed during the three year studies that the males are always in a large number compared to that of female. The sex ratio being 1:10 female: male. Multiple male frogs are seen in amplexus with a single female (Fig 2.21). There are no secondary sexual characters in *Rana alticola*. The only way to distinguish between male and female is the size where the female is always bigger than the male.

Combat: Combat behavior is seen in *Rana alticola* where unpaired males attack amplexant males that females have actively chosen and, if they are larger than amplexant males, can displace them. Males always greatly outnumber females and attempt to dislodge one another from the backs of females (Fig 2.21). In the observation, male-male competition takes the form of caller-satellite associations, in which a male calling from a call site or breeding territory is attended closely by one or more non-calling males that seek to intercept and mate with females as they approach the calling male (Fig 2.22).

Amplexing pairs and clutch size: Sexual dimorphism is pronounced where the female is almost double the size of male. As mentioned earlier in chapter I, the size of the female is always larger than that of the male. The sex ratio in this species is always male-biased. Only forty amplexing pairs were collected during the entire study period i.e, from 2005 to 2007. This may be due to the fact that females are very rare compared to males at the breeding site. The ratio of male to female is 10:1. There is a very clear-cut sexual dimorphism in *Rana alticola* (Fig 2. 20) where the females are always larger than the males. The Snout Vent Length (SVL) of mature

females were 39.08 ± 3.27 mm, where $n = 40$ (range 32.33 – 46.89 mm) and that of Snout Vent Length (SVL) of mature males were 51.33 ± 4.25 mm, where $n = 40$ (range 41.9 – 60.92 mm). The clutch size ranges from 1002 – 2018 numbers with mean 1321.1 ± 316.54 , where $n = 40$. There is no correlation between SVL of female and the clutch size ($r=0.021$; $p=0.09$) (Table 2.6).

Oviposition: Amplexus in this species is axillary, where the male holds the female at her arm pit (Fig 2.20). The female starts depositing the eggs after some hours from the time of amplexus and amplexus can continue up to 24 hours. The eggs are deposited in multiple clutches and the colour of the egg is light brown. The egg measures about 1.2 to 1.5 mm in diameter. Oviposition sites of *Rana alticola* were found amongst leaf litter along the edge of the water bodies. The eggs are deposited in the stagnant water as well as flowing water but attached to vegetation and are always submerged in the water (Fig 2.23 and 2.24) and since the eggs are laid in multiple clutch, it is impossible to know the clutch size in the field. So in order to know the clutch size, the amplexing pairs were brought to the laboratory and when the female oviposits (Fig 2.25 and 2.26), the numbers of eggs were counted and recorded. In the laboratory, when the female oviposits it was observed that the female oviposits a single egg (Fig 2.27) or multiple egg (Fig 2.28).

During the breeding season which coincides with the rainy season from late June to October, the water level at the three study sites increases (Fig 2.11a, 2.12a and 2.13a) resulting in strong water currents which in turn carry away the eggs oviposited by the frog around the breeding sites. This may be the reason why tadpoles of *Rana alticola* are found in almost every water body whereas the adults are very rare. The breeding season continues till late October when the level of

water at the breeding site decreases (Fig 2.11b, 2.12b & 2.13b), the water currents also decreases with the decrease in rainfall. Since the breeding season of *Rana alticola* starts from June and continue till October, the ecological factors of the breeding sites during the breeding periods i.e., from June to October (2005 to 2007) at all the three study sites are highlighted. It was observed that rain is the primary extrinsic factor affecting the time of the breeding activity of *Rana alticola* where the minimum rainfall recorded was 67 mm and the maximum rainfall recorded was 704 mm (Table 2.1; Fig 2.29, 2.30 & 2.31). The minimum relative humidity recorded was 24% and the maximum relative humidity recorded was 98% (Table 2.2; Fig 2.31, 2.32 & 2.33). The air temperature was relatively warmer during the breeding season which ranged from 16°C to 35°C (Table 2.3; Fig 2.35, 2.36 & 2.37). The minimum water temperature recorded was 13°C while the maximum water temperature recorded was 26°C (Table 2.4; Fig 2.38, 2.39 & 2.40). Minimum pH recorded was 6.5 and the maximum was 7.5 (Table 2.5; Fig 2.41, 2.42 & 2.43).

From November 2005 to March 2006 and November 2006 to March 2007, the adults were very rare in all the three study sites. The adults, if encountered during the non breeding season, were found in bamboo stumps present around the study sites (Fig 2.44). During the non breeding season, there is decrease in the level of the water at all the three study sites (Fig 2.11b, 2.12b & 2.13b). A large number of tadpoles are found during this period. During these periods (i.e., from November 2005 to March 2006 and November 2006 to March 2007), in all the three study sites, there is decrease in the amount of rainfall where the rainfall ranged from 1.2 mm to 57.1 mm (Table 2.1; Fig 2.29, 2.30 & 2.31). The minimum relative humidity recorded was 26% and the maximum recorded was 93% (Table 2.2; Fig 2.32, 2.33 & 2.34).

Simultaneously, there was decrease in the air temperature with minimum recorded at 12°C and maximum recorded at 32°C (Table 2.3; Fig 2.35, 2.36 & 2.37). The minimum water temperature recorded was 10°C and the maximum recorded was 24°C (Table 2.4; Fig 2.38, 2.39 & 2.40). Minimum pH recorded was 6.4 and the maximum was 7.6 (Table 2.5; Fig 2.41, 2.42 & 2.43).

From April to May (2005 to 2007), only juveniles of *Rana alticola* were seen around the study sites, and no breeding activities were seen during these months. No adults were seen at all but a large number of metamorphosed froglets were observed during these periods (Fig 2.45 a, b, c & d). The metamorphosed froglets were seen in a huge number on the rock boulders (Fig 2.45 a), among the vegetations in the water (Fig 2.45 b), on bamboo stumps (Fig 2.45 c) and between rock crevices (Fig 2.45 d) present around the study sites. During these periods (i.e., from April to May, 2005, 2006 and 2007), in all the three study sites rainfall ranged from 49 mm to 462.1 mm (Table 2.1; Fig 2.29, 2.30 & 2.31). The relative humidity ranged from 26% to 98% (Table 2.2; Fig 2.32, 2.33 & 2.34). The air temperature also increases ranging from 15 °C to 33°C (Table 2.3; Fig 2.35, 2.36 & 2.37). There is also increase in the water temperature ranging from 12°C to 26°C (Table 2.4; Fig 2.38, 2.39 & 2.40). The pH ranged from 6.4 to 6.9 (Table 2.5; Fig 2.41, 2.42 & 2.43).

Histology of the gonads: Since the adults were seen only during the breeding season, the gonads of both the sexes during the breeding season were studied. The testes of *Rana alticola* are paired and internal. They are present just above the dorsally placed kidneys and remain suspended by a double fold of peritoneum known as the mesorchium. This mesentery surrounds each testis and is continuous with the peritoneal epithelium which covers the ventral face of each

kidney and lines the entire body cavity. The testis is whitish in colour and ovoid in shape (Fig. 2.46). Whereas the ovary of *Rana alticola* is paired, multi-lobed organs, attached to the dorsal body wall by a double-layered extension of the peritoneum known as the mesovarium. The peritoneum continues around the entire ovary as the theca externa. The ovary of the female are found in the same relative position as the testis of the male but the peritoneum extends from the dorso-mesial wall rather than from the kidneys, as in the male (Fig 2.47).

The histological study of the testis under a compound microscope revealed that during the breeding period, the seminiferous tubule were clearly distinct and appeared more or less rounded in shape, and large in size. There is presence of spermatozoa in the seminiferous tubule which were observed to be slender and filamentous. Different stages of spermatogonial cells, primary and secondary spermatocytes were also observed during this phase. Mature spermatozoa occur in a cluster with their tail extending into the lumen of the tubules (Fig 2.48).

The size of the ovary during the breeding period varies depending on the maturity of the ova in the ovary and there are several lobes on each side of the ovary. Both young and mature oocytes were found to be present during the breeding period and the size of the oocytes ranged between 0.2 and 1.5 mm in diameter. Oocytes which ranged between 0.2 and 1.1 mm in diameter were classified as young oocytes and those between 1.2 and 1.5 mm in diameter as mature oocytes (Fig 2.49).

Ecological factors at the three study sites during the study periods from the year 2005 to 2007:

Rainfall: Monthly average rainfall was recorded during the study periods from 2005 to 2007 at the three study sites. At study site I (i.e., Sairang), in the year 2005, the rainfall ranged from 10 mm in the month of February to 594 mm in the month of July. No rainfall was received in the month of December. In the year 2006, the minimum rainfall recorded was 6 mm in the month of March and the maximum recorded was 426 mm in the month of July. No rainfall was received in the months of January, February and December. In the year 2007, the minimum rainfall recorded was 24 mm in the month of March and the maximum recorded was 530 mm in the month of September. No rainfall was received in the months of January and December. At study site II (i.e., Herhse), in the year 2005, the rainfall ranged from 14 mm in the month of February to 704 mm in the month of July. No rainfall was received in the months of January, November and December. In the year 2006, the minimum rainfall recorded was 1.2 mm in the month of March and the maximum recorded was 673.1 mm in the month of June. No rainfall was received in the months January, February and December. In the year 2007, the minimum rainfall recorded was 11 mm in the month of November and the maximum recorded was 611.4 mm in the month of September. No rainfall was received in the months January and December. And at study site III (i.e., Tamdil), in the year 2005, the rainfall ranged from 13 mm in the month of January to 565.4 mm in the month of July. No rainfall was received in the months February and December. In the year 2006, the minimum rainfall recorded was 83.6 mm in the month of April and the maximum recorded was 609.8 mm in the month of June. No rainfall was received in the months of January,

February, March, November and December. In the year 2007, the minimum rainfall recorded was 39 mm in the month of March and the maximum recorded was 522 mm in the month of June. No rainfall was received in the months of January and December (Table 2.1; Fig 2.29, 2.30 & 2.31).

During the breeding season (June to October) from 2005 to 2007, at study site I the rainfall ranged from 67 mm to 594 mm. At study site II, rainfall ranged from 121 mm to 704 mm. At study site III, rainfall ranged from 113.2 mm to 609.8 mm (Table 2.1; Fig 2.29, 2.30 & 2.31).

During the non breeding season (i.e., from November 2005 to May 2006 and November 2006 to May 2007), there is decrease in the amount of rainfall. During this period, at study site I, rainfall ranged from 6 mm to 423 mm. No rainfall was observed in December 2005 and January, February and December 2006 and January 2007. At study site II, rainfall ranged from 1.2 mm to 462. No rainfall was observed in November and December 2005, January, February and December 2006 and January 2007. At study site III, rainfall ranged from 37.3 mm to 460.6 mm. No rainfall was observed in December 2005, January, February, March, November and December 2006 and January 2007 (Table 2.1; Fig 2.29, 2.30 & 2.31).

Relative Humidity: Monthly average relative humidity was recorded during the study periods from 2005 to 2007 at the three study sites. At study site I (i.e., Sairang), the relative humidity ranged from 24% to 98% in the year 2005, 26% to 94% in the year 2006 and 24% to 97% in the year 2007. At study site II (i.e., Herhse), the relative humidity ranged from 44% to 98% in the year 2005, 42% to 95% in the year 2006 and 50% to 98% in the year 2007. And at study site III (i.e., Tamdil), the relative humidity ranged from 41% to 98% in the year 2005, 45% to

95% in the year 2006 and 50% to 97% in the year 2007 (Table 2.2; Fig 2.32, 2.33 & 2.34).

During the breeding season (June to October) from 2005 to 2007, at study site I the relative humidity ranged from 24% to 98%. At study site II, the relative humidity ranged from 47% to 98%. At study site III, the relative humidity ranged from 60% to 98% (Table 2.2; Fig 2.32, 2.33 & 2.34).

During the non breeding season (i.e., from November 2005 to May 2006 and November 2006 to May 2007), at study site I the relative humidity ranged from 26% to 93%. At study site II, the relative humidity ranged from 42% to 98%. At study site III, the relative humidity ranged from 45% to 91% (Table 2.2; Fig 2.32, 2.33 & 2.34).

Air Temperature: Monthly average air temperature was recorded during the study periods from 2005 to 2007 at the three study sites. At study site I, the air temperature ranged from 12°C to 35°C in the year 2005, 14°C to 31°C in the year 2006 and 13°C to 32°C in the year 2007. At study site II, the air temperature ranged from 15°C to 35°C in the year 2005, 14°C to 34°C in the year 2006 and 14°C to 33°C in the year 2007. And at study site III, the air temperature ranged from 17°C to 32°C in the year 2005, 18°C to 31°C in the year 2006 and 17°C to 32°C in the year 2007 (Table 2.3; Fig 2.35, 2.36 & 2.37).

During the breeding season (June to October) from 2005 to 2007, at study site I the air temperature ranged from 16°C to 35°C. At study site II the air temperature ranged from 17°C to 35°C. At study site III the air temperature ranged from 19°C to 32°C (Table 2.3; Fig 2.35, 2.36 & 2.37).

During the non breeding season (i.e., from November 2005 to May 2006 and November 2006 to May 2007), at study site I the air temperature ranged from 12°C to 32°C. At study site II the air temperature ranged from 14°C to 31°C. At study site III the air temperature ranged from 17°C to 31°C (Table 2.3; Fig 2.35, 2.36 & 2.37).

Water Temperature: Monthly average water temperature was recorded during the study periods from 2005 to 2007 at the three study sites. At study site I, the water temperature ranged from 11°C to 25°C in the year 2005, 11°C to 25°C in the year 2006 and 10°C to 26°C in the year 2007. At study site II, the water temperature ranged from 11°C to 25°C in the year 2005, 12°C to 26°C in the year 2006 and 12°C to 25°C in the year 2007. And at study site III, the water temperature ranged from 11°C to 24°C in the year 2005, 12°C to 23°C in the year 2006 and 11°C to 23°C in the year 2007 (Table 2.4; Fig 2.38, 2.39 & 2.40).

During the breeding season (June to October) from 2005 to 2007, at study site I the water temperature ranged from 13°C to 26°C. At study site II the water temperature ranged from 14°C to 25°C. At study site III the water temperature ranged from 13°C to 23°C (Table 2.4; Fig 2.38, 2.39 & 2.40).

During the non breeding season (i.e., from November 2005 to May 2006 and November 2006 to May 2007), at study site I the water temperature ranged from 10°C to 25°C. At study site II the water temperature ranged from 11°C to 26°C. At study site III the water temperature ranged from 11°C to 23°C (Table 2.4; Fig 2.38, 2.39 & 2.40).

pH: Monthly average pH was recorded during the study periods from 2005 to 2007 in the three study sites. At study site I, the pH ranged from 6.7 to 7.8 in the year 2005, 6.6 to 7.2 in the year 2006 and 6.6 to 7.4 in the year 2007. At study site

II, the pH ranged from 6.4 to 7 in the year 2005, 6.5 to 7 in the year 2006 and 6.6 to 7 in the year 2007. And at study site III, the pH ranged from 6.7 to 6.9 in the year 2005, 6.8 to 7.2 in the year 2006 and 6.5 to 7.1 in the year 2007 (Table 2.5; Fig 2.41, 2.42 & 2.43).

During the breeding season (June to October) from 2005 to 2007, at study site I the pH ranged from 6.7 to 7.5. At study site II the pH ranged from 6.5 to 7. At study site III the pH ranged from 6.6 to 7 (Table 2.5; Fig 2.41, 2.42 & 2.43).

During the non breeding season (i.e., from November 2005 to May 2006 and November 2006 to May 2007), at study site I the pH ranged from 6.6 to 7.6. At study site II the pH ranged from 6.4 to 7. At study site III the pH ranged from 6.5 to 7.2 (Table 2.5; Fig 2.41, 2.42 & 2.43).

During the three year survey, it was found that rainfall was relatively high during the breeding season (i.e. from June to October). The air and water temperatures were also comparatively high during this period. The relative humidity and pH were more or less the same throughout the year.

DISCUSSION

Present findings indicate that *Rana alticola* breed both in the lotic ecosystem (Sairang and Herhse) as well as lentic (Tamdil), a wetland ecosystem. A similar condition is also seen in the case of *Bufo celebensis* which breed in streams in south-east Sulawesi (Gillespie *et al.*, 2004) as well as pond habitats in northern Sulawesi (Leong and Chou, 2000). From the present investigation, it has been observed that Tamdil, a wetland ecosystem provides a good habitat for breeding site of *Rana alticola*. Wetlands provide critical habitat for a wide variety of amphibian species. However, many amphibian species also require terrestrial habitat adjacent to wetlands to complete their life cycle (Semlitsch, 1998). During the non breeding season, most pond-breeding amphibians live in the terrestrial habitat surrounding a pond. Adults migrate to the pond during favorable weather conditions to breed and mating as well as oviposition usually occurs in the pond, and then adults return to their terrestrial habitat. After hatching, the aquatic larvae feed, grow, and develop until metamorphosis, after which they immigrate as juveniles to terrestrial habitats. This behavior has also been observed and recorded in the case of *Rana alticola*. Amphibian movements around the breeding habitat can be part of normal foraging behavior within an individual's home range (Gibbons and Bennett 1974), or repeated movements to the pond to deposit additional clutches of eggs (Wells 1976; Perrill and Daniels 1983). The terrestrial habitat adjacent to the breeding site provides food and shelter throughout the prolonged breeding season. Patterns of habitat use and community organization in stream breeding frog assemblages has received relatively little attention worldwide (Emerson & Inger 1992, Eterovick & Sazima 2000, Inger 1969, Inger & Voris 1993, Lips 1996, 1998; Parris & McCarthy 1999, Ovaska 1991,

Utsunomiya *et al.*, 1983, Williams & Hero 1998). The focus upon pond-breeding communities possibly reflects the studies of lowland neotropical assemblages, where there is a predominance of non- stream breeding species (Crump 1971). Inger (1969) and Inger & Voris (1993) have reported the only detailed studies of a stream-breeding frog community in South- East Asia. Despite the high diversity of anuran communities in the tropics, basic information on habitat use and reproductive patterns are available for relatively few tropical anuran assemblages (Aichinger 1987, Berry 1964, Crump 1974, Donnelly & Guyer 1994, Gascon 1991, Heyer 1973, Inger 1969, Trennery 1991).

It was found that *Rana alticola* emerge and starts to breed from late June where the rainfall ranges from 113.2 mm to 673.1 mm (Table 2.1; Fig 2.29, 2.30 & 2.31) during the study periods from 2005 to 2007. The breeding activity seems to be initiated by heavy rainfall which is one factor that is always associated with the amphibian breeding activity (Aichinger, 1987). Studies on the pattern of reproduction of anurans in tropical and sub tropical regions have repeatedly demonstrated that reproductive phenology is closely associated with rainfall, and this is particularly true in tropical forests that have seasonal changes in precipitation (Blair, 1961; Bowker and Bowker, 1979; Alexander *et al.*, 1979; Aichinger, 1987; Gascon, 1991; Donnelly and Guyer, 1994). Earlier studies mentioned that the reproductive activity of anurans living in seasonal environments is generally associated with the rainy periods, both in tropical climates (Hoogmoed and Gorzula, 1979, Aichinger, 1987) and in temperate ones (Banks and Beebee, 1986; Caldwell, 1987). Rain is the primary extrinsic factor affecting the time of the breeding activity for most tropical and subtropical anuran species (Haddad and Sazima, 1992;

Duellman and Trueb, 1994) as also observed in the case of *Rana alticola* in the present investigation.

Following the emergence, it was observed that the adult males of *Rana alticola* aggregate at the breeding sites and start the advertisement calls which are in chorus to attract females. When many species of anurans congregate for breeding around a single water body during breeding season, vocalization plays an important role in mate choice. Each anuran species in such mixed species chorus produces advertisement calls with distinctive temporal and spectral parameters, enabling females to identify and select conspecific males for mating (Krishna and Krishna, 2005). The advertisement call of *Rana alticola* as observed in the study is species specific, which consist of four notes and the call duration lasts for one second (Fig 2.18a). Each note consists of two to three pulses and each note lasts for 0.1 second (Fig 2.18b). The dominant frequency spread over a frequency band of 1636.52 – 2497.85 Hz. Roy *et al.*, 1998 described the call characteristics of *Rana alticola* as very rapid where each call lasts for about 0.020 s and the dominant frequency lies between 0.30 kHz to 1.57 kHz. In other ranids like *Rana curtipes*, male calls were made up of 7-8 notes and the dominant frequency was in the range of 759-1684.6 Hz (Krishna and Krishna, 2005). The advertisement call of *R. tigrina* consists of number of calls produced in series at variable intervals forming a call group. The call duration, call interval and call period vary from 164–260 ms, 330-3356 ms and 560-3520 ms respectively and the dominant frequency peak lies at 1800 Hz. The advertisement calls of *R. breviceps* last up to 2 mins and each call consists of number of calls produced in series at variable intervals forming a calls group. Each call group comprises 13–141 calls and each call consists of a single pulse group. The call

duration, call interval and call period vary from 360–592 ms, 235–1522 ms and 592–1924 ms respectively. Each call consists of 25–34 pulses without interval. The dominant frequency lies between 1100–2500 Hz (Kanamadi *et al.*, 1994)

Observations during the study indicated that besides advertisement call, *Rana alticola* also gives a distress call by both male and female adults when in captivity which differ from the advertisement call. The distress call in *Rana alticola* has two notes and the call duration lasts for two seconds (Fig 2.19a). Each note consists of six pulses and each note lasts for 0.3 second (Fig 2.19b). The dominant frequency spread over a frequency band of 1335.06 – 1421.19 Hz. This is also reported by Sazima (1975), that while a large proportions of species have a male advertisement call, a minority has, and in addition, distinct aggressive or territorial calls and encounter calls. The loud, explosive distress calls given in response to acute disturbance or grasping by a potential predator are produced by either sex or sometimes even newly metamorphosed young and are acoustically dissimilar to the advertisement calls.

Rana alticola is a prolonged breeder and exhibit a number of behaviors shared by anuran species with prolonged breeding seasons and a complex vocal repertoire which occur in several other ranids including *R. palustris* (Given, 2005), *R. catesbeiana* (Capranica, 1968; Judge and Brooks, 2001), *R. clamitans* (Wells, 1977 b, 1978), and *R. virgatipes* (Given, 1987, 1988 a, b). The advertisement call is the major factor in the courtship of *Rana alticola* which initiate the female to approach the calling male, the male will grasp the female by the armpit and amplexus will start. Amplexus in *Rana alticola* is axillary where the male grasps the female at the axilla. Most neobatrachians have axillary amplexus (Duellman and

Trueb, 1994). Sexual dimorphism is pronounced in *Rana alticola* where the female is larger than the male. This observation support in the present study supports Shine (1979) where he reported that female exhibits larger body size than males in 90% of anurans species.

During the present study, it was observed that an alternative non-calling behavioural mating tactics was seen in *Rana alticola*, where the males actively moves around the breeding area seeking females which is also seen in other species like *Bufo bufo* (Davies and Halliday 1979), *Rana sylvatica* (Howard 1980; Woolbright *et al.*, 1990) and *Bufo calamita* (Arak 1988; Tejedo 1992). In *Rana alticola* male competition to mate with the female is severe, as observed in the present study where the number of males greatly out number the females and the ratio of male to female is 10:1. Savage (1935) suggested that nuptial pads are analogous to male weapons, because of their role in competition for females, and suggested that they are evolved most highly in those species in which male-male competition is more severe. But nuptial pad is not present in *Rana alticola* even though male-male competition is severe. An interesting mating behavior is seen and recorded in *Rana alticola* where male-male competition takes the form of caller-satellite associations, in which a male calling from a call site or breeding territory is attended closely by one or more non-calling males that seek to intercept and mate with females as they approach the calling male (Fig 2. 22). Satellite males sit quietly near calling males and commonly maintain a low posture, presumably making themselves inconspicuous to the calling males, which may chase them away. Two or three satellites have been observed with one calling male in several species. Satellite males do not intercept females approaching calling males, but the satellites may take

over a calling site when a female carries the former territory-holder to an oviposition site, or satellites may replace territory-holders that have become physically weakened after maintaining their territories for several weeks (Robertson, 1981).

In *Rana alticola*, unpaired males attack amplexant males which have actively been chosen by the female and, if they are larger than amplexant males, can displace them as also seen in the case of *Ololygon rubra* (Bourne 1992). In *Rana alticola* multiple male frogs are seen in amplexus with a single female which also occur sporadically in many frog families (Byrne & Roberts 2004). *Rana alticola* males occasionally fight for the possession of female already in amplexus, and engage in protracted struggles, which is also reported in other anuran species like *Bufo bufo* (Davies and Halliday 1979), *B.americanus* (Howard 1988), *B.gutturalis* (Telford and van Sickle 1989) and *Rana sylvatica* (Howard and Kluge 1985). In the present study, it was observed that though *Rana alticola* is not an explosive breeder, both these tactics are typical of explosive breeders.

The breeding activity of *Rana alticola* starts from late June till early October. Breeding period of *Rana alticola* coincides with the monsoon from June to October, which is typical for other Indian anurans on which breeding studies have been conducted (Agarwal and Niazi, 1977; Dutta and Mohanty Hejmadi, 1976; Khan *et al.*, 1979; Mallick *et al.*, 1980; Mohanty Hejmadi and Dutta 1978, 1988; Mohanty Hejmadi *et al.*, 1979 a, b, 1980; Platt, 1986; Roy and Khare, 1978). During the breeding season from 2005 to 2007, the air temperature (Table 2.3; Fig 2.35, 2.36 & 2.37) and water temperature (Table 2.4; Fig 2.38, 2.39 & 2.40) were relatively high. As observed for other anuran species, climatic factors, especially rainfall and temperature exert a strong influence on the reproductive activity (Telford and Dyson,

1990; Silverin and Andrin 1992) and conditions of stress can even inhibit ovarian development. Also environmental variables such as humidity, temperature and photoperiod may determine anuran breeding period (Navas, 1996; Navas and Bevier, 2001, Hatano *et al.*, 2002). Similarly, other studies reported that anuran breeding biology is influenced by climatic factors like temperature and rainfall or a combination of these, both for tropical and temperate species (Duellman and Trueb 1986; Beebee 1995a).

Oviposition sites of *Rana alticola* were found amongst leaf litter along the edge of the water bodies both in the lotic and lentic ecosystem. The eggs were deposited in the stagnant water as well as flowing water but attached to vegetation and were always submerged in the water (Fig 2.23 and Fig 2.24). The frogs that oviposit in ephemeral aquatic sites are dependent upon heavy rainfall; those that utilize streams usually breed at times of little rainfall, when the water level is low and the current is slow. Similarly, this is seen in the case of *Rana alticola* where the breeding activity starts from late June where there is heavy rainfall which provides water to the breeding ephemeral aquatic sites. Breeding activity of *Rana alticola* continues till October where the rainfall is decreasing and the water level is low and current is slow. Hence, *Rana alticola* chose the leaf litter along the edge of the stream as oviposition sites. Thus, even though temperature and moisture may be sufficient throughout the year, unpredictability of oviposition sites probably restricts reproduction activity (Duellman and Trueb, 1994). Calling and oviposition site selection are important determinants of reproductive success, and therefore fitness, in anurans (Lips, 1996, Resetarits & Wilbur, 1991). The selection of a suitable oviposition site is observed in *Rana alticola* where there is no parental care and this

is of critical importance in the reproductive success of organisms that lack parental care (Murphy, 2003).

The clutch size of *Rana alticola* ranges from 1002 – 2018. There is no correlation between SVL of female and the clutch size. In *Rana tigerina* the eggs are laid in a clutch in shallow water and the clutch size ranges from 4660 to 6460 and there is no correlation between the eggs and the female size (Dutta and Mohanty-Hejmadi, 1976). *Rana cyanophlyctis* clutch ranges from 300 to 500 (Mohanty-Hejmadi and Dutta, 1978). Another interesting feature recorded in *Rana alticola*, is that the eggs are deposited in multiple clutches which is also seen in several anuran families like Hylidae; *Hyla chrysoscelis* (Godwin and Roble 1983; Ritke et al. 1992), *Hyla cinerea*, *H. regilla* and *H. gratoisa* (Perrill and Daniel 1983), *Pachymedusa dacnicolor* (Iela et al., 1986), Ranidae; *Rana clamitans* (Wells 1976), *Rana catesbeiana* (Emlen 1977), *Hyperolius marmoratus* (Telford and Dyson 1990), Bufonidae; *Bufo calamita* (Banks and Beebee 1986), Discoglossidae; *Discoglossus pictus* (Knoepffler 1962), *Bombina variegata* and *B. bombina* (Obert 1977; Kapfberger 1984); *Alytes muletensis* (Bush 1993); Dendrobatidae; *Dendrobates granuliferus* (Crump 1972). Myobatrachidae: *Crinia signifera* (Lemckert and Shine 1993). Duellman and Trueb, 1994 reported that many pond breeding anurans produce large clutches of small eggs and have low survival rates of both eggs and larvae. This may also be true for *Rana alticola*. Eggs laid in water may be in the form of large clumps representing the entire ovarian complement or the clump may represent only part of the ovarian complement as in the case of *Rana alticola* where the female deposits small parcels of eggs at different sites, here clumps of eggs are attached to sticks or vegetation in the water which serve to maintain the position of

the clutch in the pond or stream. Many studies have demonstrated that female size correlates positively with the number of eggs laid or ovarian eggs produced (Martins, 1988, Ryser, 1988, Praderio and Robinson, 1990). The same was not observed in *Rana alticola*.

The histological study of the testis under a compound microscope revealed that during the breeding period, the seminiferous tubule were clearly distinct and appeared more or less rounded in shape, and large in size. There is presence of spermatozoa in the seminiferous tubule which were observed to be slender and filamentous. Different stages of spermatogonial cells, primary and secondary spermatocytes were also observed during this phase. Mature spermatozoa occur in a cluster with their tail extending into the lumen of the tubules (Fig. 2.48). Reproductive activities of most amphibians are greatly susceptible to environmental fluctuations, thus most of them exhibit markedly seasonal testicular cycle. Rastogi *et al.*, (1978) studied various environmental influences which could alter the characteristics of internal morphology of the testis in *R. esculenta*, and found that these influences consist of rainfall, temperature and photoperiod. These factors cause cyclical external morphological changes as well as internal changes of the testis. As *Rana alticola* adults were found only during the breeding season, histological study of the testis was done only during the breeding season where different stages of spermatogonial cells, primary and secondary spermatocytes were observed. In *Rana ridibunda*, with continuous type spermatogenesis, show significantly increased number of spermatids during breeding season around April to June, while in winter, the number of spermatocytes was decreased and reach minimum in the coldest month (Loumbourdis and Kyriakopoulou-Sklavounou, 1991).

During the present study, it was found that the size of the ovary during the breeding period varies depending on the maturity of the ova in the ovary and there are several lobes on each side of the ovary. Both young and mature oocytes were found to be present during the breeding period (Fig. 2.49). Environmental temperature and day length are known to influence ovarian activity in *R. cyanophlyctis* (Pancharatna and Kulkarni 1993, Pancharatna and Patil 1997). The correlative annual changes in the ovarian mass and oviduct weight have been reported for *R. cyanophlyctis* (Pancharatna and Saidapur 1985) and other tropical anurans such as, *Rana tigrina* (Pramoda and Saidapur, 1984), *B. melanostictus* (Kanamadi *et al.*, 1989) and *Polypedates maculatus* (Kanamadi and Jirankali, 1991). As in the case of *Rana alticola*, the histophysiological study during the spawning period shows both young and mature oocytes which has also been conducted for *R. cyanophlyctis* (Suvarnalatha *et al.*, 1975).

Table 2.3: Average air temperature at study sites I, II and III during 2005 to 2007.

| Months | 2005 | | | | | | | | | 2006 | | | | | | | | | 2007 | | | | | | | | | | | |
|--------|------|-----|----|-----|-----|----|-----|-----|----|------|-----|----|-----|-----|----|-----|-----|----|------|-----|----|-----|-----|----|-----|-----|----|-----|-----|----|
| | I | | | II | | | III | | | I | | | II | | | III | | | I | | | II | | | III | | | | | |
| | Min | Max | | Min | Max | | Min | Max | | Min | Max | | Min | Max | | Min | Max | | Min | Max | | Min | Max | | Min | Max | | Min | Max | |
| Jan | 13 | 28 | 16 | 28 | 18 | 27 | 14 | 24 | 14 | 28 | 18 | 27 | 13 | 27 | 16 | 28 | 19 | 28 | 15 | 25 | 15 | 30 | 18 | 27 | 17 | 30 | 14 | 27 | 21 | 31 |
| Feb | 14 | 32 | 17 | 29 | 19 | 28 | 16 | 25 | 16 | 29 | 20 | 26 | 17 | 30 | 17 | 32 | 19 | 28 | 17 | 30 | 14 | 27 | 21 | 31 | 18 | 29 | 15 | 31 | 24 | 29 |
| Mar | 14 | 32 | 18 | 33 | 22 | 31 | 18 | 30 | 17 | 32 | 19 | 28 | 17 | 30 | 17 | 32 | 19 | 28 | 17 | 30 | 14 | 27 | 21 | 31 | 18 | 29 | 15 | 31 | 24 | 29 |
| Apr | 15 | 32 | 20 | 31 | 23 | 32 | 19 | 29 | 20 | 30 | 22 | 31 | 18 | 29 | 20 | 30 | 22 | 31 | 18 | 29 | 15 | 31 | 24 | 29 | 18 | 29 | 15 | 31 | 24 | 29 |
| May | 16 | 33 | 21 | 33 | 24 | 33 | 22 | 30 | 21 | 31 | 21 | 27 | 20 | 28 | 17 | 32 | 22 | 31 | 20 | 28 | 17 | 32 | 22 | 31 | 21 | 30 | 18 | 30 | 22 | 32 |
| Jun | 16 | 32 | 22 | 32 | 23 | 31 | 22 | 31 | 22 | 34 | 22 | 28 | 21 | 31 | 18 | 30 | 22 | 32 | 21 | 31 | 18 | 30 | 22 | 32 | 21 | 30 | 18 | 30 | 22 | 32 |
| Jul | 16 | 27 | 21 | 28 | 24 | 32 | 21 | 30 | 22 | 32 | 23 | 30 | 20 | 30 | 22 | 31 | 23 | 29 | 21 | 32 | 21 | 32 | 24 | 28 | 21 | 32 | 21 | 32 | 24 | 28 |
| Aug | 20 | 28 | 24 | 29 | 22 | 29 | 22 | 31 | 23 | 33 | 20 | 27 | 21 | 32 | 21 | 32 | 21 | 32 | 20 | 31 | 23 | 33 | 22 | 31 | 20 | 31 | 23 | 33 | 22 | 31 |
| Sep | 24 | 35 | 21 | 35 | 23 | 28 | 20 | 31 | 24 | 32 | 22 | 28 | 20 | 31 | 24 | 32 | 22 | 28 | 20 | 31 | 23 | 33 | 22 | 31 | 17 | 30 | 17 | 31 | 23 | 29 |
| Oct | 22 | 35 | 20 | 34 | 19 | 29 | 17 | 30 | 23 | 33 | 21 | 27 | 17 | 30 | 23 | 33 | 21 | 27 | 15 | 29 | 16 | 29 | 19 | 28 | 15 | 29 | 16 | 29 | 19 | 28 |
| Nov | 18 | 32 | 16 | 31 | 19 | 24 | 14 | 29 | 22 | 31 | 19 | 27 | 15 | 29 | 16 | 29 | 19 | 28 | 15 | 29 | 16 | 29 | 19 | 28 | 15 | 29 | 16 | 29 | 19 | 28 |
| Dec | 12 | 28 | 15 | 30 | 17 | 26 | 14 | 28 | 18 | 28 | 18 | 29 | 15 | 23 | 15 | 28 | 17 | 27 | 15 | 23 | 15 | 28 | 17 | 27 | 15 | 23 | 15 | 28 | 17 | 27 |

Table 2.4: Average water temperature at study sites I, II and III during 2005 to 2007

| Months | 2005 | | | | | | 2006 | | | | | | 2007 | | | | | |
|--------|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|------|-----|-----|-----|-----|-----|
| | I | | II | | III | | I | | II | | III | | I | | II | | III | |
| | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max | Min | Max |
| Jan | 10 | 16 | 11 | 15 | 12 | 17 | 11 | 14 | 12 | 22 | 12 | 18 | 10 | 14 | 12 | 18 | 11 | 14 |
| Feb | 12 | 16 | 12 | 17 | 13 | 18 | 13 | 15 | 12 | 24 | 13 | 18 | 12 | 14 | 13 | 17 | 13 | 15 |
| Mar | 12 | 19 | 14 | 22 | 12 | 20 | 14 | 22 | 14 | 23 | 13 | 21 | 13 | 16 | 13 | 16 | 12 | 19 |
| Apr | 14 | 23 | 14 | 23 | 14 | 24 | 15 | 23 | 15 | 25 | 17 | 23 | 12 | 23 | 14 | 22 | 14 | 21 |
| May | 15 | 22 | 15 | 24 | 15 | 24 | 18 | 25 | 17 | 26 | 16 | 21 | 15 | 25 | 16 | 23 | 16 | 21 |
| Jun | 15 | 24 | 17 | 25 | 17 | 23 | 19 | 25 | 19 | 23 | 18 | 22 | 17 | 26 | 18 | 25 | 17 | 23 |
| Jul | 15 | 25 | 16 | 24 | 17 | 23 | 17 | 25 | 17 | 24 | 19 | 23 | 16 | 23 | 17 | 24 | 18 | 21 |
| Aug | 14 | 24 | 17 | 23 | 16 | 21 | 18 | 23 | 18 | 19 | 16 | 23 | 19 | 24 | 16 | 23 | 20 | 23 |
| Sep | 13 | 18 | 15 | 19 | 15 | 21 | 14 | 21 | 14 | 17 | 15 | 20 | 15 | 19 | 15 | 20 | 16 | 21 |
| Oct | 13 | 16 | 14 | 17 | 13 | 19 | 13 | 19 | 15 | 19 | 16 | 21 | 14 | 19 | 13 | 20 | 14 | 21 |
| Nov | 12 | 15 | 13 | 18 | 11 | 18 | 12 | 16 | 14 | 18 | 13 | 18 | 13 | 17 | 12 | 18 | 13 | 18 |
| Dec | 10 | 12 | 11 | 16 | 12 | 17 | 12 | 15 | 12 | 17 | 12 | 15 | 12 | 15 | 12 | 16 | 12 | 17 |

Table 2.5: Average pH at study sites I, II and III during 2005 to 2007.

| Months | 2005 | | | 2006 | | | 2007 | | |
|--------|------|-----|-----|------|-----|-----|------|-----|-----|
| | I | II | III | I | II | III | I | II | III |
| Jan | 7.4 | 7.0 | 6.8 | 7.1 | 7 | 6.9 | 7.3 | 6.9 | 6.7 |
| Feb | 7.8 | 6.9 | 6.6 | 7.2 | 6.9 | 7.0 | 7.1 | 6.7 | 7.1 |
| Mar | 6.8 | 6.6 | 6.8 | 6.7 | 6.8 | 6.7 | 6.8 | 7.0 | 6.6 |
| Apr | 6.7 | 6.4 | 6.7 | 6.6 | 6.7 | 6.9 | 6.6 | 6.9 | 6.8 |
| May | 6.8 | 6.7 | 6.9 | 6.8 | 6.8 | 6.8 | 6.7 | 6.8 | 6.5 |
| Jun | 6.9 | 6.5 | 6.6 | 7.1 | 6.9 | 6.9 | 7.1 | 6.6 | 6.6 |
| Jul | 7.5 | 6.8 | 6.6 | 6.9 | 7.0 | 6.8 | 6.9 | 7.0 | 6.7 |
| Aug | 7.1 | 6.8 | 6.9 | 7.1 | 6.7 | 6.9 | 7.4 | 6.8 | 6.7 |
| Sep | 7.5 | 6.9 | 6.7 | 7.1 | 6.8 | 6.7 | 7.3 | 6.9 | 6.8 |
| Oct | 6.9 | 7 | 6.8 | 6.7 | 6.5 | 7 | 6.7 | 6.7 | 6.9 |
| Nov | 7.6 | 6.9 | 6.9 | 6.8 | 6.6 | 7.2 | 6.9 | 6.8 | 7.1 |
| Dec | 7.2 | 6.4 | 6.8 | 7.2 | 6.9 | 6.8 | 7.1 | 6.9 | 6.8 |

Table 2.6 SVL of male and female with the clutch size of *Rana alticola*

| Sl. No. | SVL of female (mm) | SVL of male (mm) | Clutch size |
|---------|--------------------|------------------|-----------------|
| 1 | 53.88 | 38.74 | 1181 |
| 2 | 55.47 | 41.84 | 1200 |
| 3 | 49.12 | 37.26 | 1500 |
| 4 | 51.45 | 38.14 | 1800 |
| 5 | 52.85 | 37.67 | 1000 |
| 6 | 54.2 | 37.47 | 1952 |
| 7 | 55.42 | 41.5 | 2018 |
| 8 | 50.92 | 38.0 | 1210 |
| 9 | 53.7 | 38.35 | 1825 |
| 10 | 60.92 | 39.62 | 1025 |
| 11 | 41.9 | 36.25 | 1112 |
| 12 | 45.8 | 37.74 | 1901 |
| 13 | 49.98 | 36.48 | 1800 |
| 14 | 48 | 36.88 | 1420 |
| 15 | 46.28 | 36.3 | 1122 |
| 16 | 45.38 | 35.22 | 1091 |
| 17 | 42.9 | 36.82 | 1421 |
| 18 | 51.26 | 35.92 | 1162 |
| 19 | 48.22 | 35.11 | 1232 |
| 20 | 51.2 | 44.23 | 1145 |
| 21 | 49.08 | 36.4 | 1023 |
| 22 | 48.34 | 37.9 | 1109 |
| 23 | 51.2 | 41.22 | 1256 |
| 24 | 54.4 | 44.6 | 1098 |
| 25 | 46.0 | 36.9 | 1190 |
| 26 | 56.8 | 42.9 | 1009 |
| 27 | 47.98 | 37.34 | 1892 |
| 28 | 51.23 | 42.8 | 1290 |
| 29 | 48.34 | 37.55 | 1190 |
| 30 | 52.34 | 38.89 | 1120 |
| 31 | 56.22 | 39.12 | 1003 |
| 32 | 48.90 | 32.33 | 1134 |
| 33 | 56.0 | 44.92 | 1004 |
| 34 | 52.89 | 39.88 | 1389 |
| 35 | 56.97 | 43.67 | 1782 |
| 36 | 48.92 | 46.89 | 1056 |
| 37 | 55.34 | 36.7 | 1002 |
| 38 | 56.9 | 42.8 | 1679 |
| 39 | 49.68 | 36.9 | 1119 |
| 40 | 56.90 | 43.9 | 1382 |
| Mean | 51.33 ± 4.25 | 39.08 ± 3.27 | 1321.1 ± 316.54 |

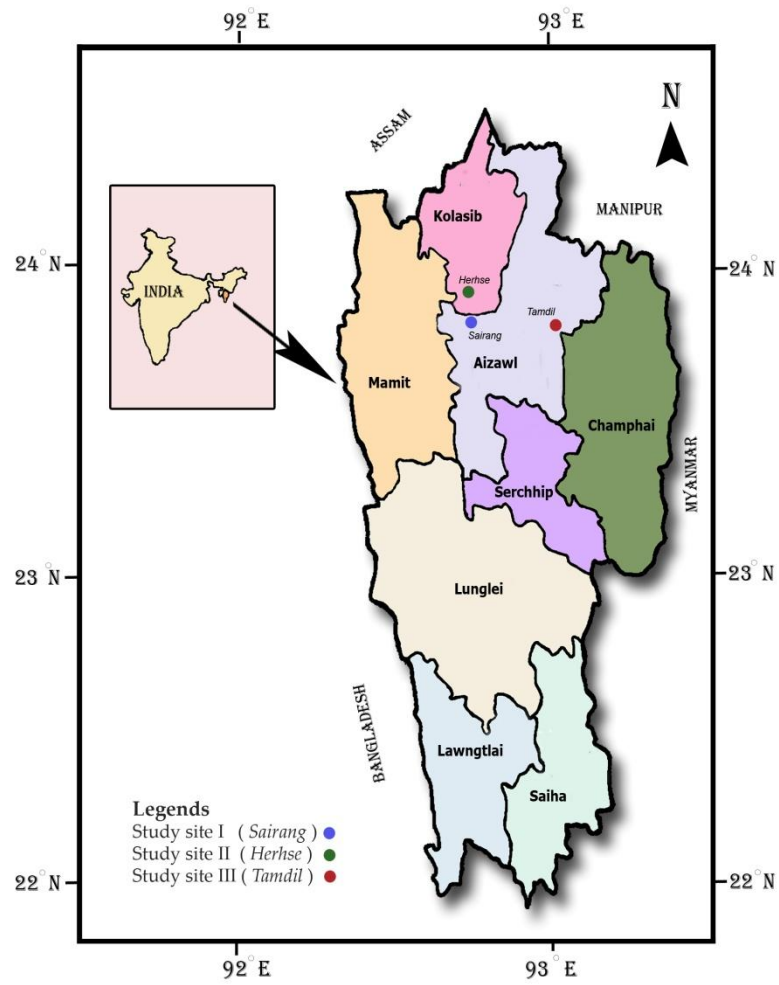


Fig 2.1: Map of Mizoram showing the three study sites

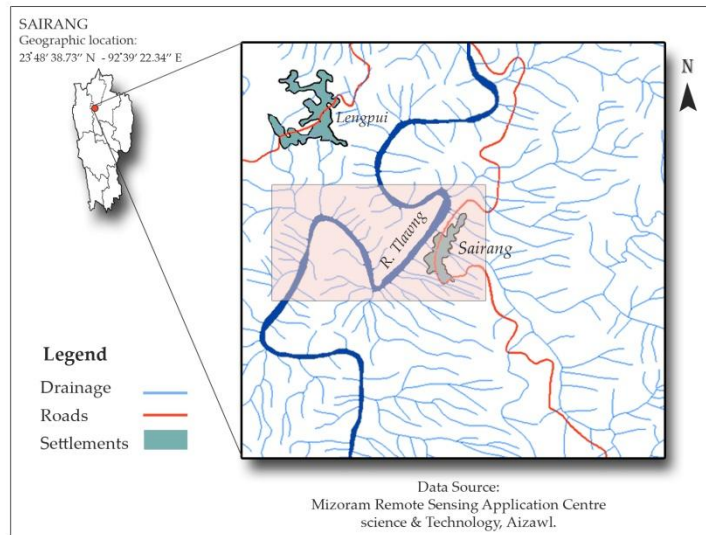


Fig 2.2 : Study site I (Sairang)



Fig 2.3 : Satellite image of study site I (Sairang)



Fig 2.4 : Some vegetation found in and around study site I (Sairang River)

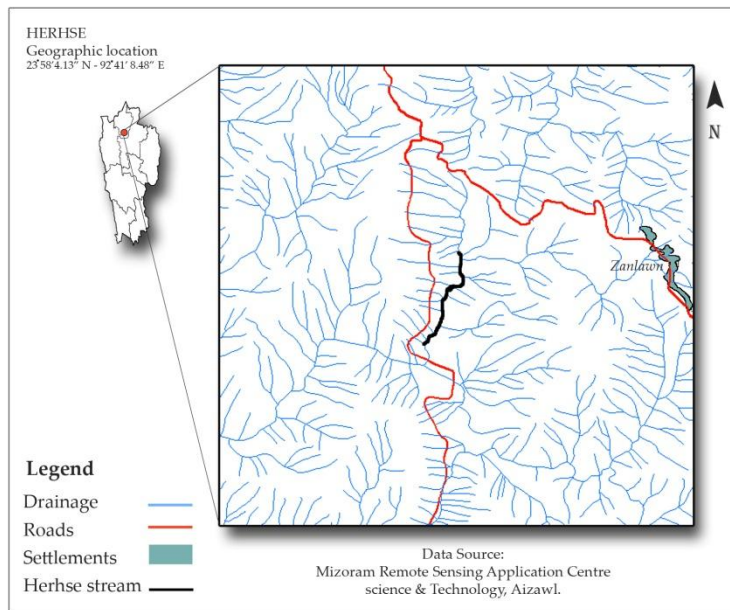


Fig 2.5 : Study site II (Herhse)



Fig 2.6 : Satellite image of study site II (Herhse)

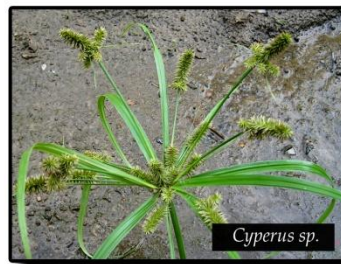


Fig 2.7 : Some vegetation found in and around study site II (Herhse Stream)

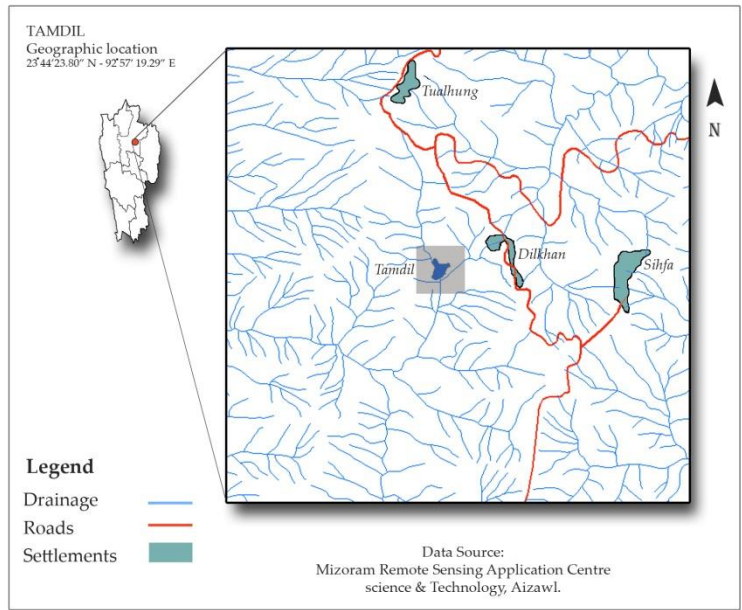


Fig 2.8 : Study site III (Tamdil)



Fig 2.9 : Satellite image of study site III (Tamdil)

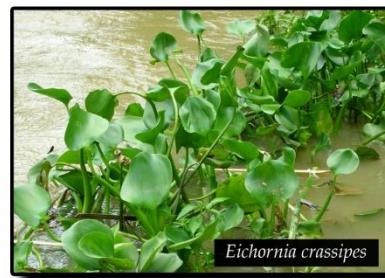
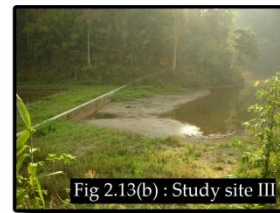
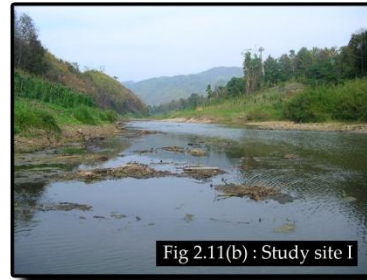
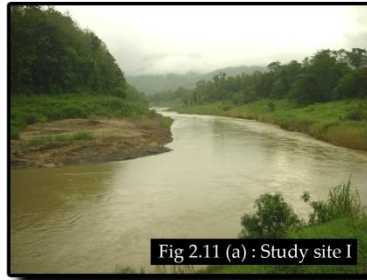


Fig 2.10 : Some vegetations found in and around study site III (Tamdil)



a = study sites I, II and III during the breeding season
b = study sites I, II and III during the non breeding season



Fig 2.14 : Female emerging



Fig 2.15 : Males emerging



Fig 2.16 : Calling male in the field



Fig 2.17 : Gravid female in the field

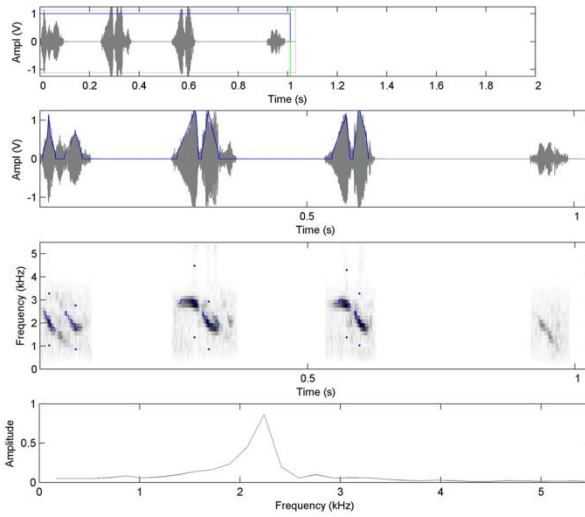


Fig 2.18a : Detailed oscillogram of full note of the advertisement call of *Rana alticola*

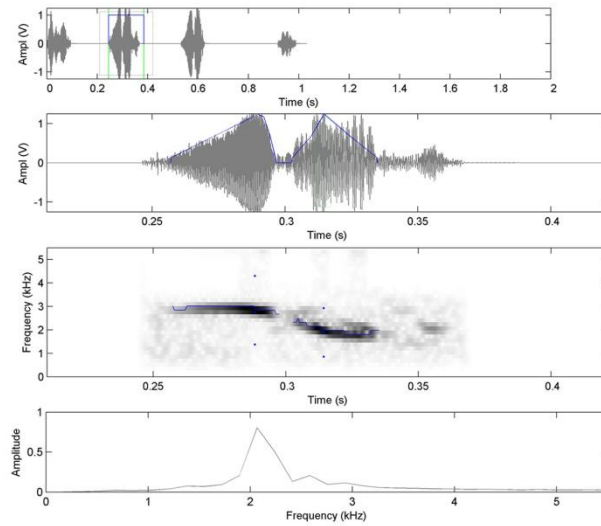


Fig 2.18b : Detailed oscillogram of one note of the advertisement call of *Rana alticola*

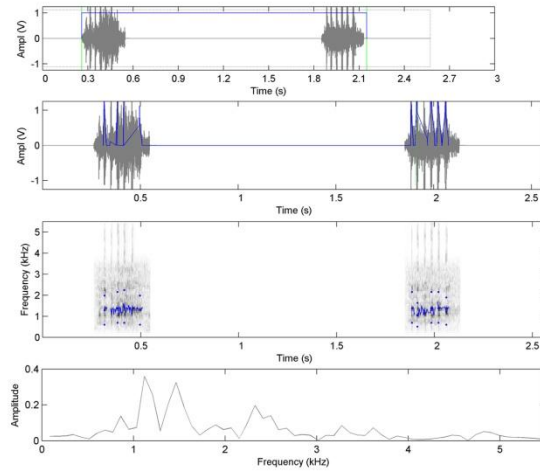


Fig 2.19a : Detailed oscillogram of full note of the distress call of *Rana alticola*

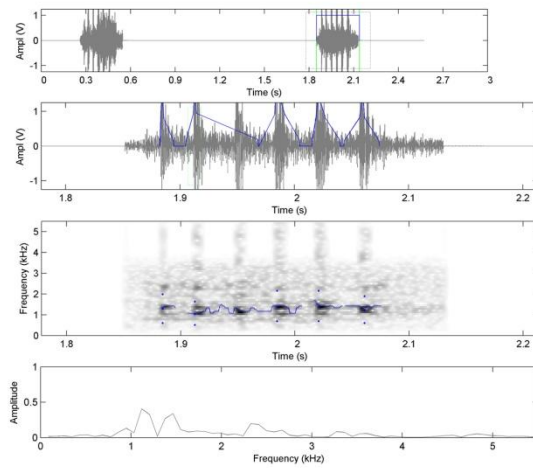


Fig 2.19b : Detailed oscillogram of one note of the distress call of *Rana alticola*



Fig 2. 20 : Axillary amplexus



Fig 2.21 : Combat behavior ; Males fighting for a single female.



Fig 2.22 : Satellite male



Fig 2.23 : *Rana alticola* laying eggs in the field



Fig 2.24 : After egg laying in the field



Fig 2.25 : *Rana alticola* laying eggs in the laboratory.



Fig 2.26 : After egg laying in the laboratory



Fig 2.27 : Laying of single egg



Fig 2.28 : Laying of multiple eggs

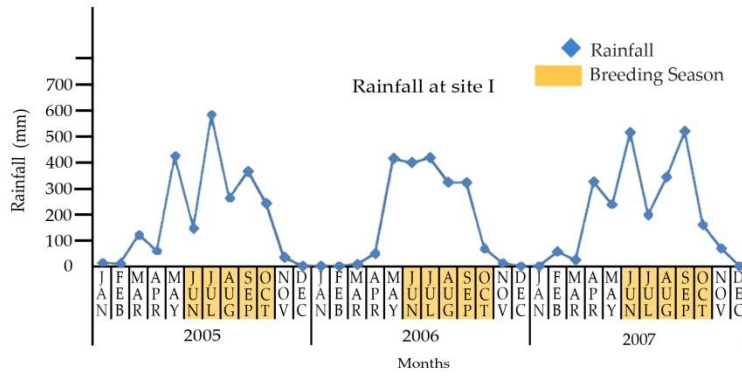


Fig 2.29 : Monthly variation of rainfall at study site I

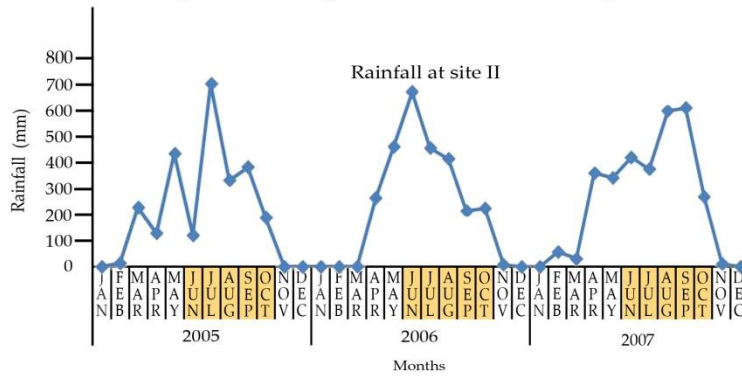


Fig 2.30 : Monthly variation of rainfall at study site II

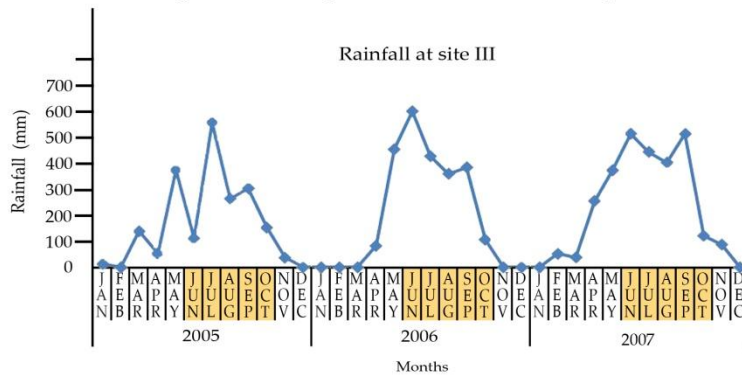


Fig 2.31 : Monthly variation of rainfall at study site III

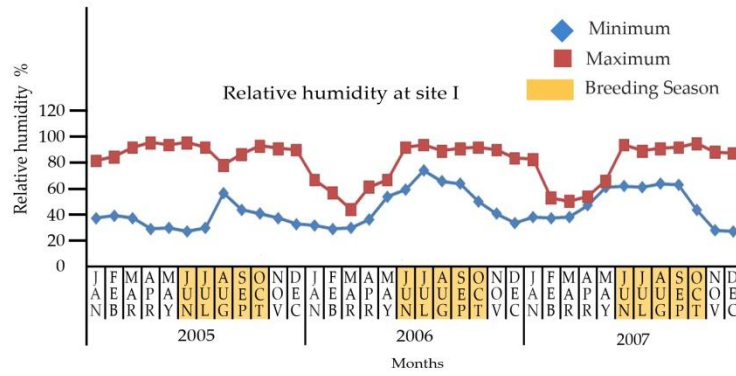


Fig 2.32 : Monthly variation of relative humidity at study site I

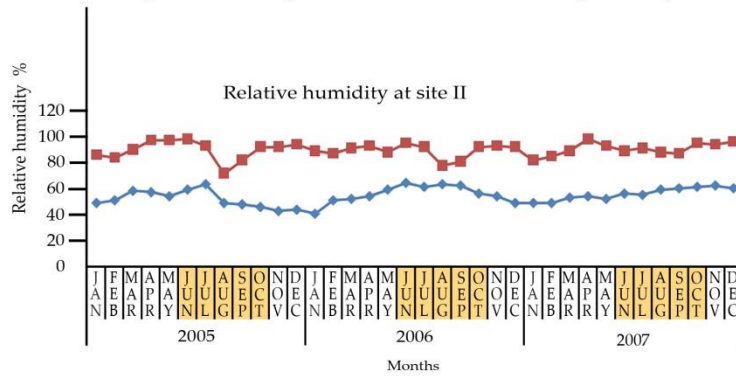


Fig 2.33 : Monthly variation of relative humidity at study site II

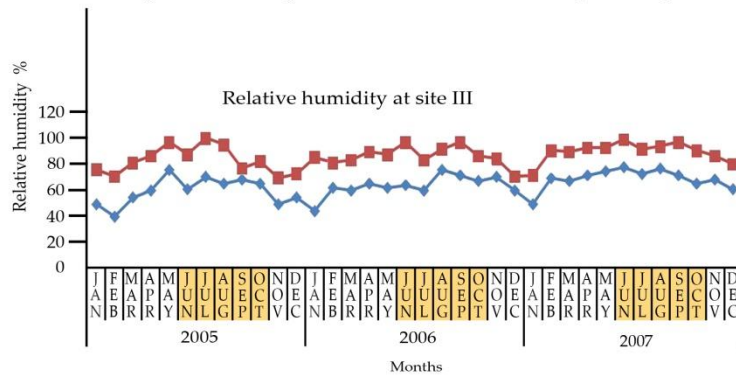


Fig 2.34 : Monthly variation of relative humidity at study site III

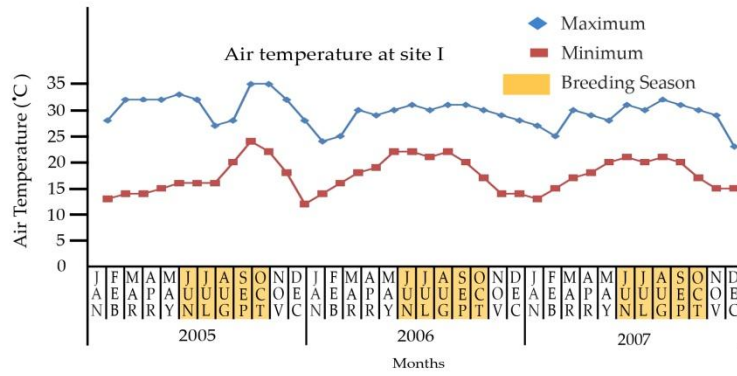


Fig 2.35 : Monthly variation of Air temperature at study site I

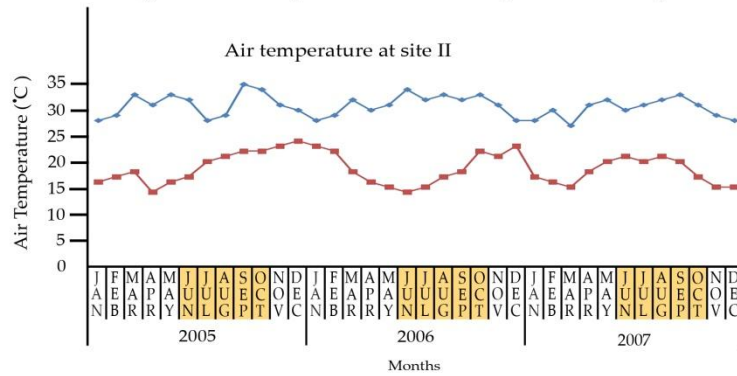


Fig 2.36 : Monthly variation of Air temperature at study site II

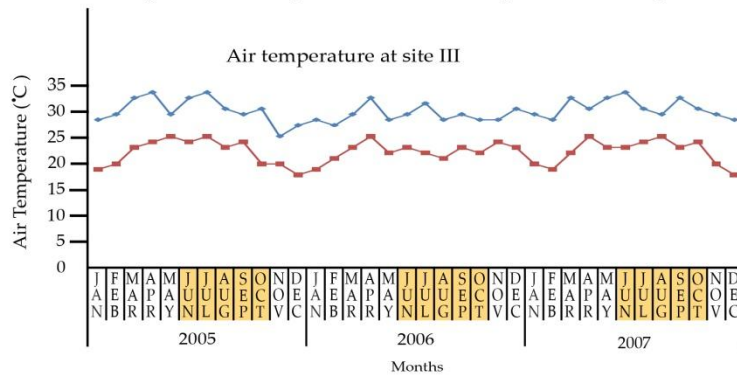


Fig 2.37 : Monthly variation of Air temperature at study site III

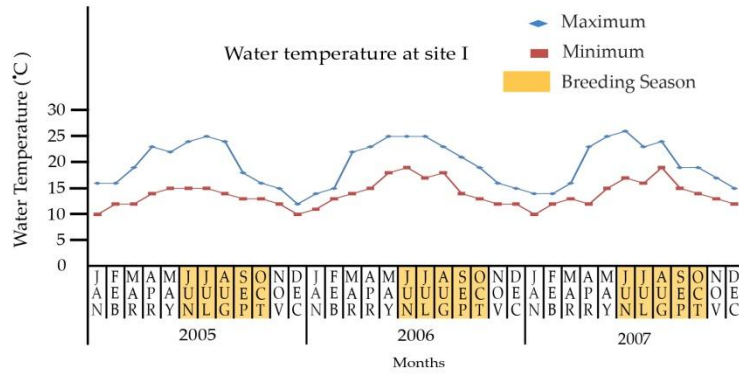


Fig 2.38 : Monthly variation of Water temperature at study site I

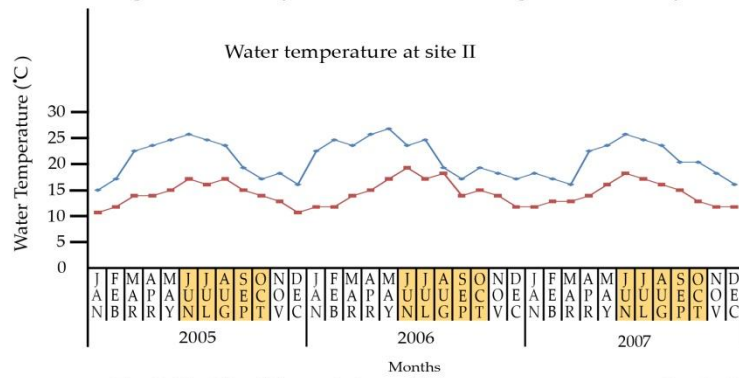


Fig 2.39 : Monthly variation of Water temperature at study site II

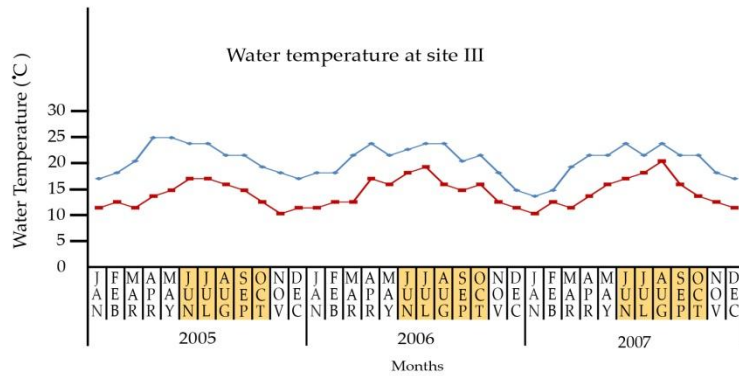


Fig 2.40 : Monthly variation of Water temperature at study site III

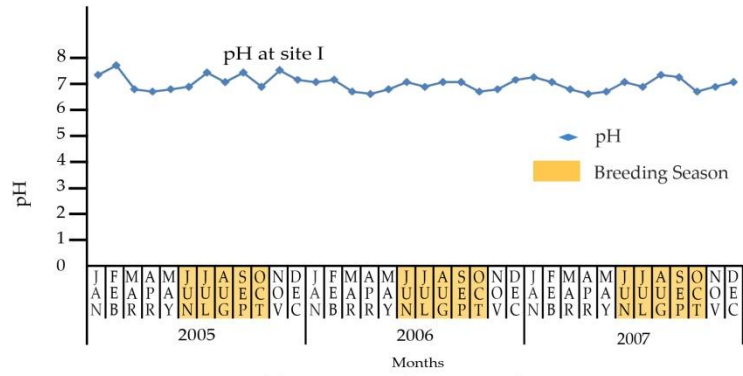


Fig 2.41 : Monthly variation of pH at study site I

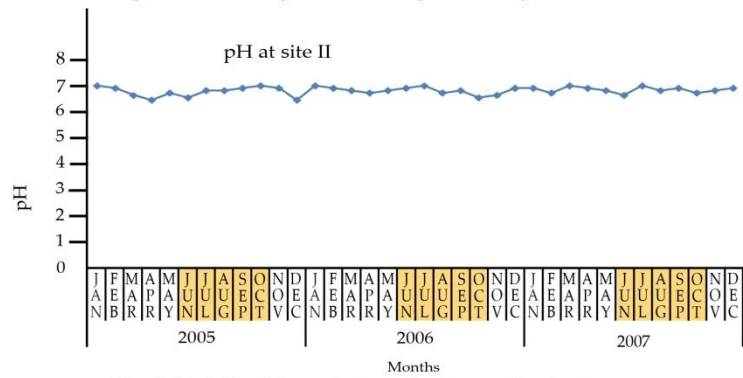


Fig 2.42 : Monthly variation of pH at study site II

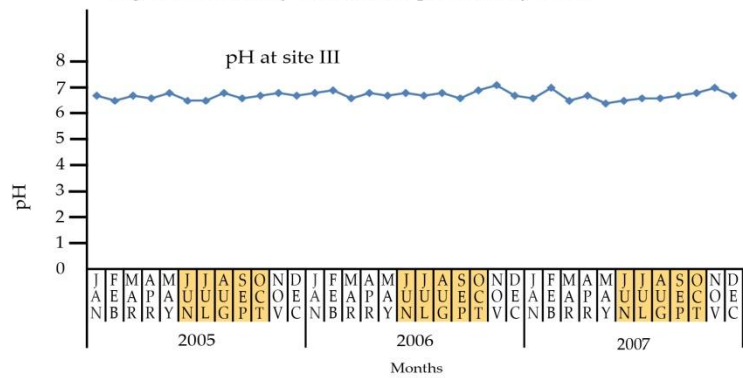


Fig 2.43 : Monthly variation of pH at study site III



Fig 2.44 : *Rana alticola*
inside a bamboo stump.



Fig 2.45a : Froglets seen on rocks

Fig 2.45b : Froglets seen
on leaves



Fig 2.45c : Froglets seen
on bamboo

Fig 2.45d : Froglets seen inside
rock crevices

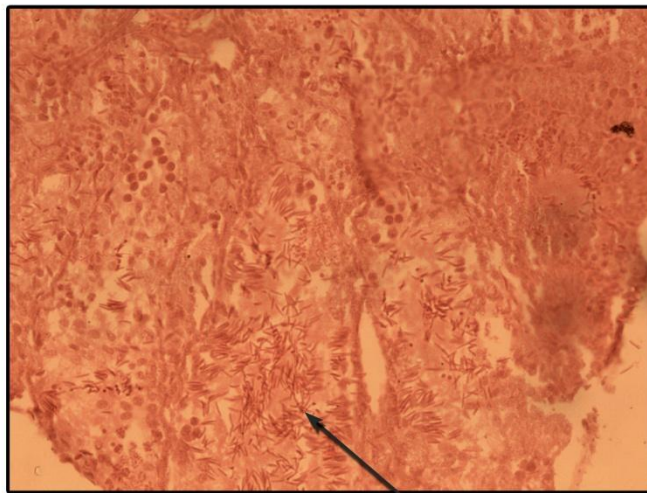




Fig 2.46 : Testis of adult male of *Rana alticola*

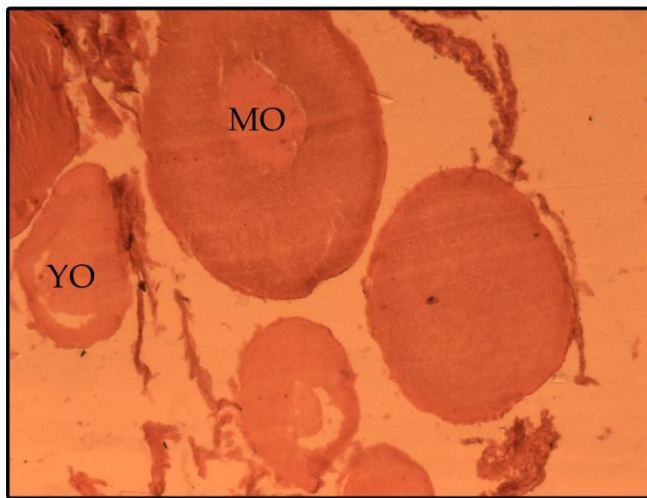


Fig 2.47 : Eggs of a gravid female of *Rana alticola*



Mature spermatozoa

Fig 2.48 : Cross section of testis of *Rana alticola* during the breeding season (x 20)



YO = Young oocyte; MO = Mature oocyte

Fig 2.49 : Cross section of the ovary of *Rana alticola* during the breeding season (x 20)

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 Trueb, 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.

REVIEW OF LITERATURE

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*

(Roy and Khare, 1978), *Rana (Euphlyctis) cyanophlyctis* (Mohanty-Hejmadi and Dutta, 1978).

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 desiccation (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 starts 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. 47 a 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 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 infra-caudal gland; another at the beginning of dorsal fin, the supra-caudal 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 bud 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 35mm 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 65.36mm (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 61mm. 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 pipiens* (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

| Sl.No. | Stage | Age | Size (mm) | Hind limb (mm) |
|--------|-----------------------------|----------------|---------------|----------------|
| 1. | Fertilization | 0 hr | 1.2 – 1.5 | |
| 2. | Gray Crescent | 30 mins | “ | |
| 3. | 2-cell | 5 hrs | “ | |
| 4. | 4-cell | 6 hrs 30 mins | “ | |
| 5. | 8-cell | 8 hrs 11 mins | “ | |
| 6. | 16-cell | 10 hrs 25 mins | “ | |
| 7. | 32-cell | 12hrs 33 mins | “ | |
| 8. | Mid Cleavage | 14 hrs 50 mins | “ | |
| 9. | Late Cleavage | 16 hrs 50 mins | “ | |
| 10. | Dorsal Lip | 44 hrs | “ | |
| 11. | Mid gastrula | 48 hrs 25 mins | 1.3 - 1.6 | |
| 12. | Late Gastrula | 58 hrs 35 mins | “ | |
| 13. | Neural Plate | 4 days | 1.45 - 1.75 | |
| 14. | Neural Fold | 5 days | 1.5 - 1.8 | |
| 15. | Rotation | 6 days 10 hrs | 1.5 - 1.8 | |
| 16. | Neural Tube | 7 days 20 hrs | 1.7 - 2 | |
| 17. | Tail Bud | 9 days | 1.8 - 2.1 | |
| 18. | Muscular Response | 12 days | 2.3 - 2.5 | |
| 19. | Heart Beat | 13 days 20 hrs | 3.5 - 3.6 | |
| 20. | Tail Elongation | 16 days 15 hrs | 3.8 - 4 | |
| 21. | Cornea Transparent | 18 days 10 hrs | 4.5 - 5 | |
| 22. | Tail Fin Circulation | 20 days | 6.5 - 7 | |
| 23. | Operculum covers gill bases | 24 days | 7.5 - 8 | |
| 24. | Operculum closes on right | 28 days | 8 - 8.5 | |
| 25. | Spiracles Forms | 31 days | 11 - 30 | |
| 26. | L < ½D | 51 days | 34.1 – 64.6 | 0.5 |
| 27. | L = ½D | 59 days | 39.8 – 67.7 | 0.7 |
| 28. | L = D | 64 days | 48 – 71.7 | 0.9 |
| 29. | L = 1½D | 72 days | 48.1 – 73.63 | 1.5 |
| 30. | L = 2D | 79 days | 49 – 75.63 | 1.9 |
| 31. | Foot Paddle | 88 days | 51.5 – 76.2 | 2 |
| 32. | Indentation 4-5 | 99 days | 54.07 – 77.63 | 2.5 |
| 33. | Indentation 3-4 | 108 days | 52.86 – 81.63 | 3.2 |
| 34. | Indentation 2-3 | 115 days | 60.2 – 91.3 | 3.5 |
| 35. | Indentation 1-2 | 120 days | 51.92 – 89.82 | 3.85 |
| 36. | Toes 3-5 Separated | 127 days | 71 – 90.7 | 4.5 |
| 37. | All Toes Separated | 133 days | 61.1 – 86.5 | 6 |
| 38. | Metatarsal tubercles | 145 days | 52 – 95.72 | 6.8 |
| 39. | Sub-articular patches | 160 days | 56.7 – 83.94 | 8.9 |
| 40. | Vent tube present | 175 days | 82 – 93 | 13.7 |
| 41. | Fore Limbs Visible | 187 days | 52.9 – 91.64 | 34 |
| 42. | Fore Limbs emerge | 194 days | 69.4 – 92.2 | 41 |
| 43. | Tail Atrophies | 196 days | 35 – 83.5 | 45 |
| 44. | Tail Greatly Reduced | 203 days | 48.3 – 93.8 | 65.36 |
| 45. | Tail Stub | 208 days | 37 – 61 | 72.96 |
| 46. | Metamorphosis Complete | 215 days | 23 – 39.1 | 68 |

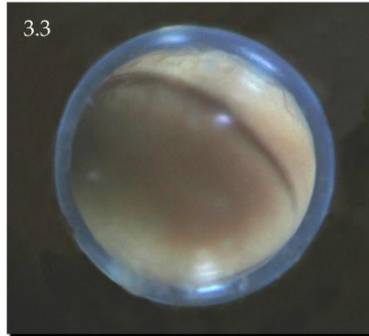
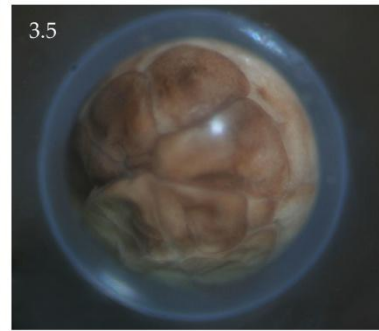
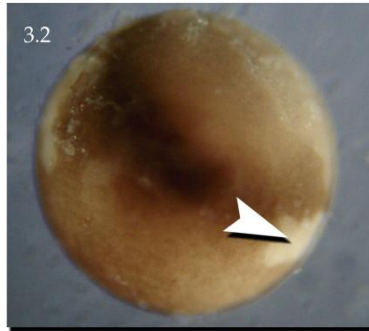
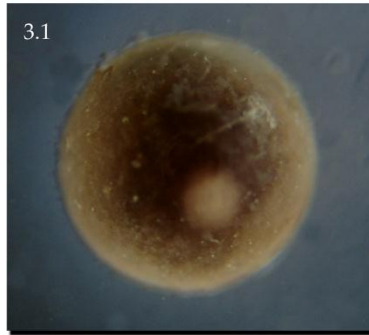


Fig 3.1 : Stage 1 - Fertilization

Fig 3.4 : Stage 4 - Four cell stage

Fig 3.2 : Stage 2 - Gray crescent

Fig 3.5 : Stage 5 - Eight cell stage

Fig 3.3 : Stage 3 - Two cell stage

Fig 3.6 : Stage 6 - Sixteen cell stage

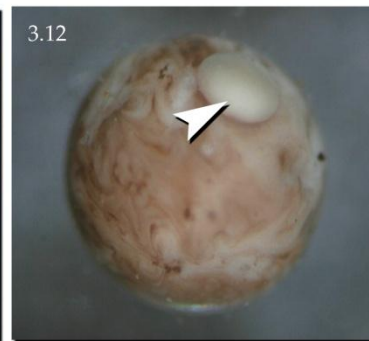
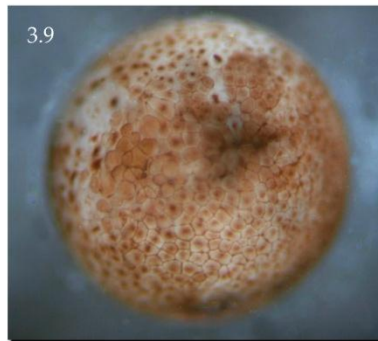
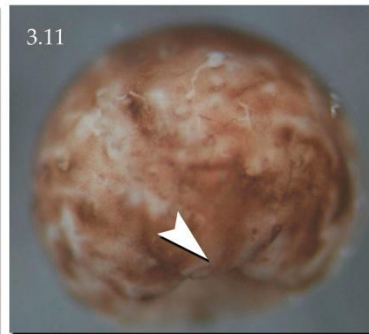
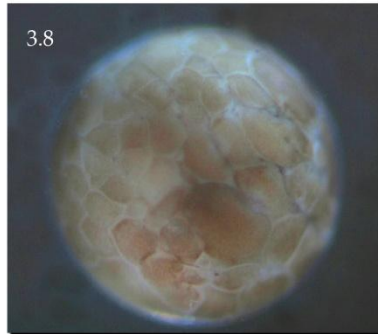
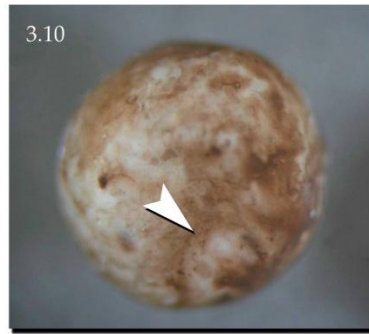
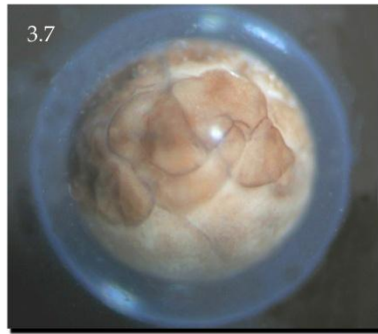


Fig 3.7 : Stage 7 - Thirty two cell stage

Fig 3.10 : Stage 10 - Dorsal lip

Fig 3.8 : Stage 8 - Mid cleavage

Fig 3.11 : Stage 11 - Mid Gastrula

Fig 3.9 : Stage 9 - Late cleavage

Fig 3.12 : Stage 12 - Late Gastrula

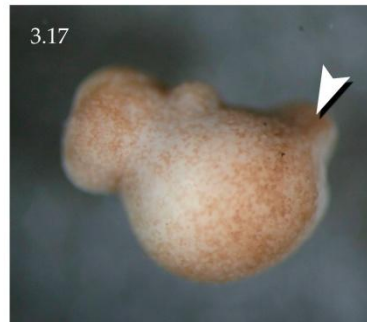
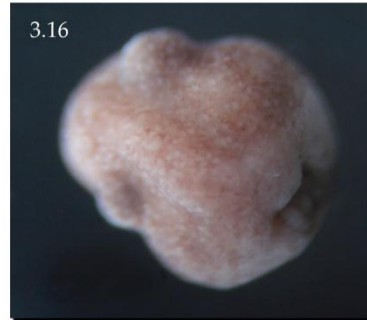
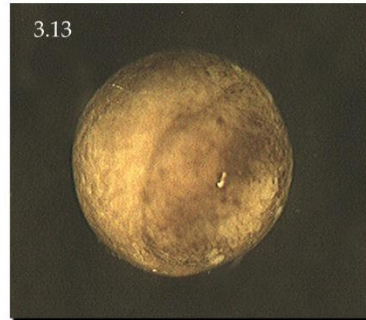


Fig 3.13 : Stage 13 - Neural plate

Fig 3.16 : Stage 16 - Neural tube

Fig 3.14 : Stage 14 - Neural folds

Fig 3.17 : Stage 17 - Tail bud

Fig 3.15 : Stage 15 - Rotation

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]



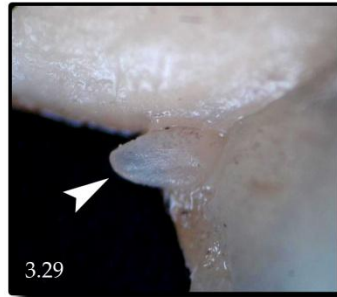
Fig 3.25b : Stage 25 - Dorsal view



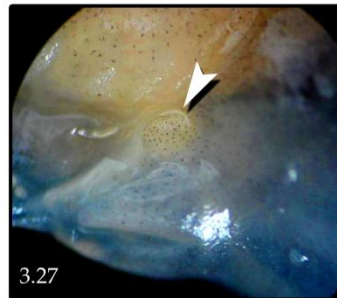
Tadpole Hind Limb Bud Development



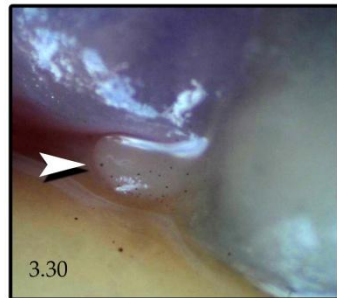
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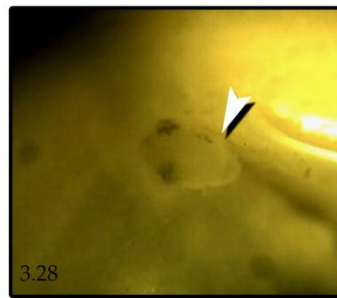
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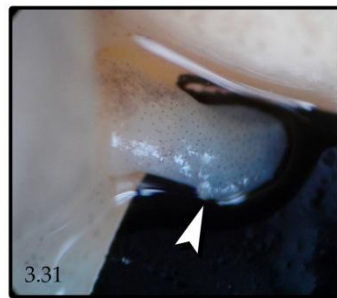
3.27



3.30



3.28



3.31

Fig 3.26 : Stage 26 - $L < \frac{1}{2} D$

Fig 3.27 : Stage 27 - $L \geq \frac{1}{2} D$

Fig 3.28 : Stage 28 - $L \geq D$

Fig 3.29 : Stage 29 - $L \geq 1\frac{1}{2} D$

Fig 3.30 : Stage 30 - $L = 2 D$

Fig 3.31 : Stage 31 - Foot paddle



Tadpole toe Differentiation and Development

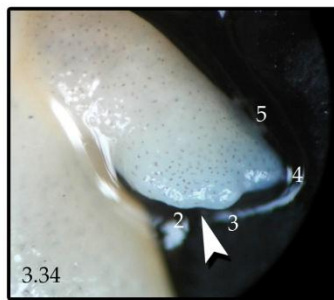
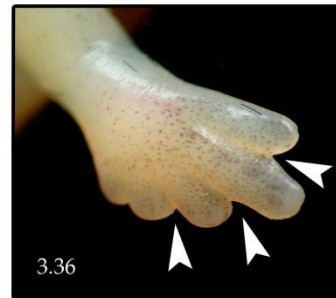
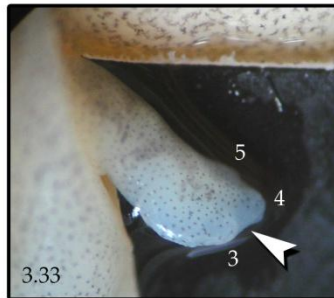
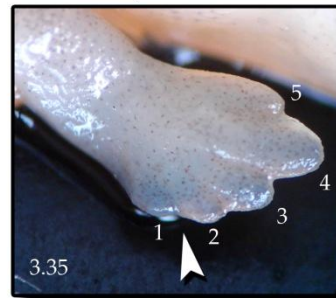
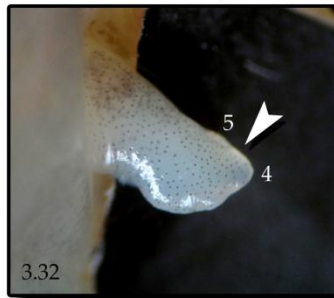


Fig 3.32 : Stage 32 - Indentation 4- 5

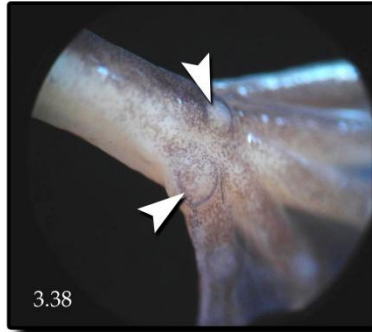
Fig 3.35 : Stage 35 - Indentation 1- 2

Fig 3.33 : Stage 33 - Indentation 3- 4

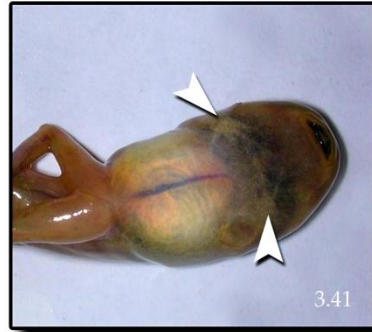
Fig 3.36 : Stage 36 - Toes 3- 5 separated

Fig 3.34 : Stage 34 - Indentation 2- 3

Fig 3.37 : Stage 37 - All toes separated



3.38



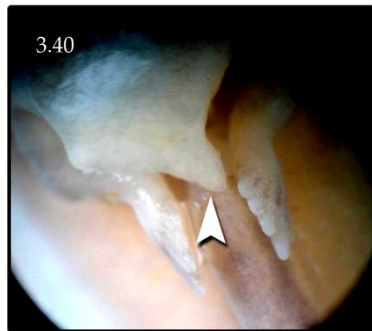
3.41



3.39



3.42a



3.40



3.42b

Fig 3.38 : Stage 38 - Metatarsal tubercle Fig 3.41 : Stage 41 - Fore limbs visible

Fig 3.39 : Stage 39 - Subarticular patches

Fig 3.42a : Stage 42 - Left forelimb emerge first

Fig 3.40 : Stage 40 - Vent tube present

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.

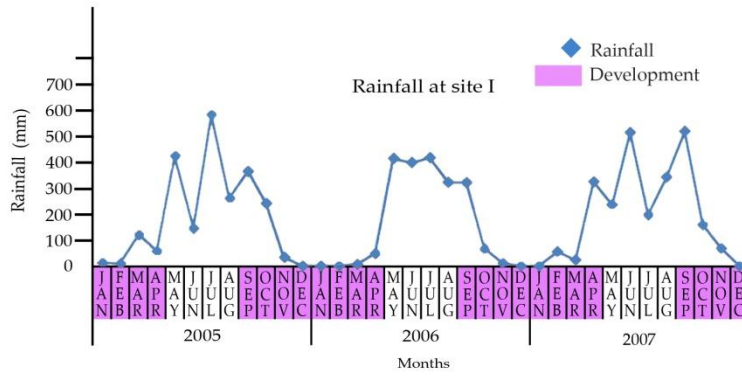


Fig 3.48: Monthly variation of rainfall at study site I

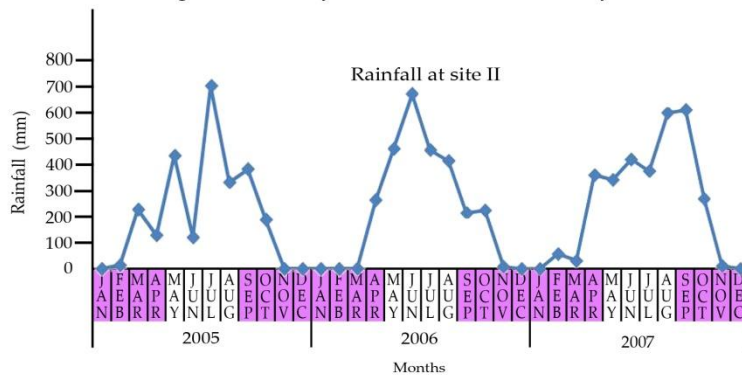


Fig 3.49: Monthly variation of rainfall at study site II

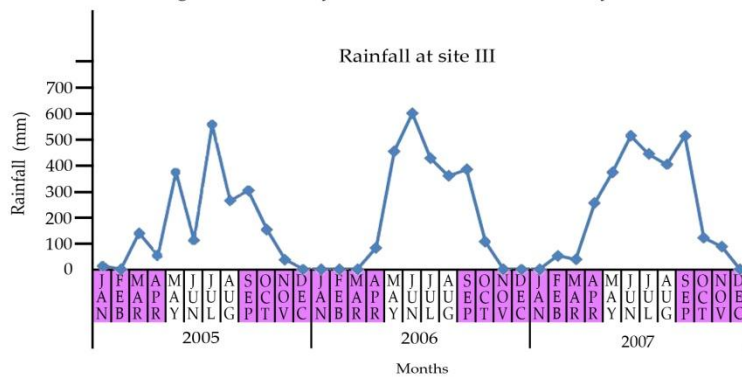


Fig 3.50: Monthly variation of rainfall at study site III

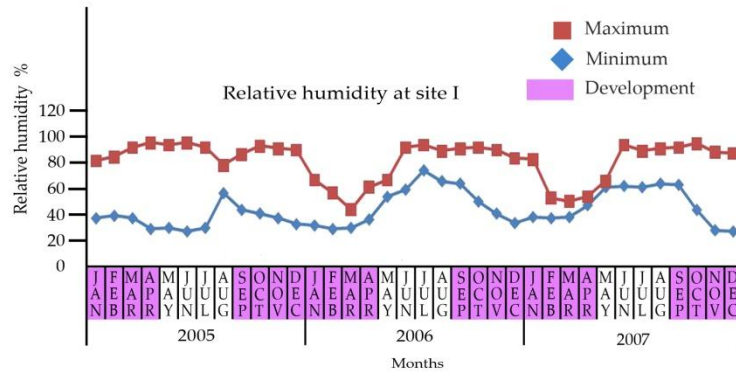


Fig 3.51: Monthly variation of relative humidity at study site I

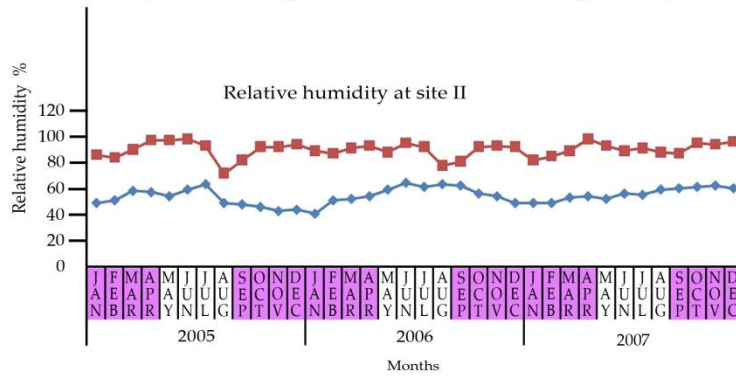


Fig 3.52: Monthly variation of relative humidity at study site II

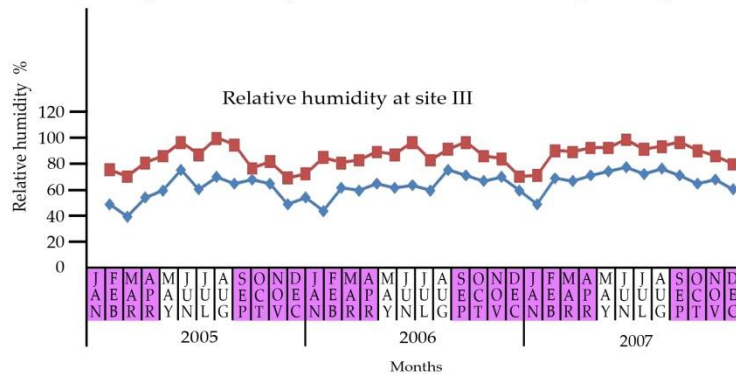


Fig 3.53: Monthly variation of relative humidity at study site III

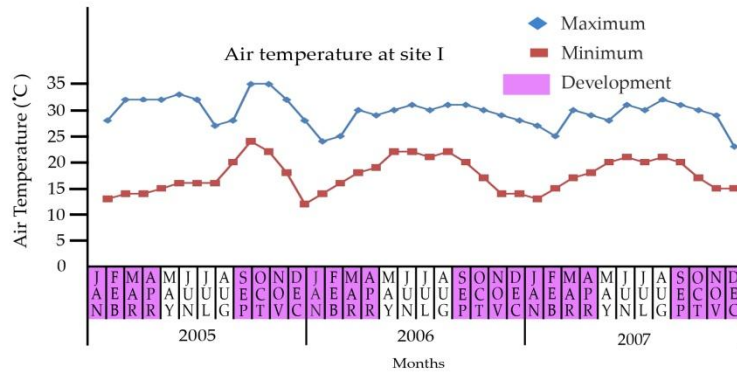


Fig 3.54: Monthly variation of Air temperature at study site I

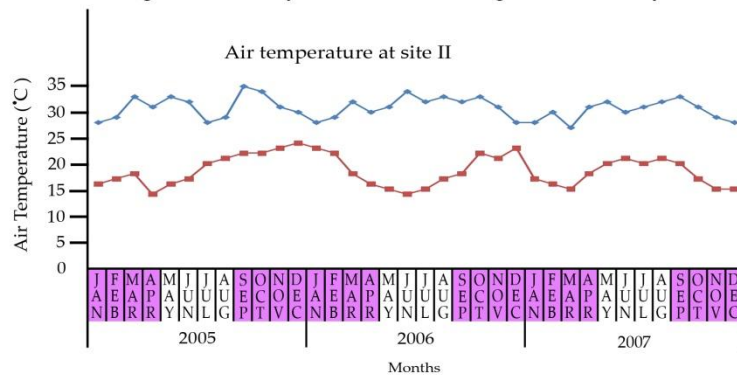


Fig 3.55: Monthly variation of Air temperature at study site II

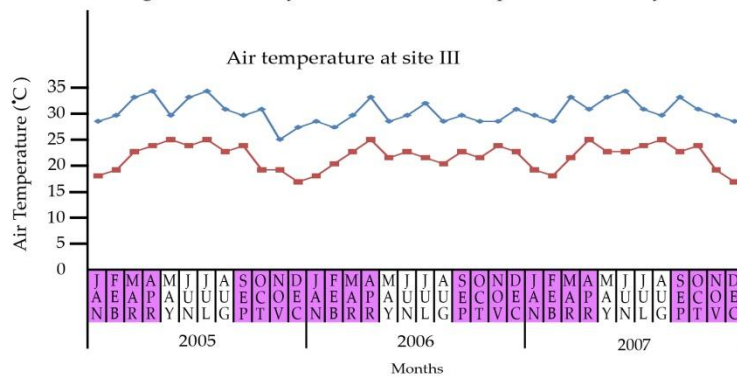


Fig 3.56: Monthly variation of Air temperature at study site III

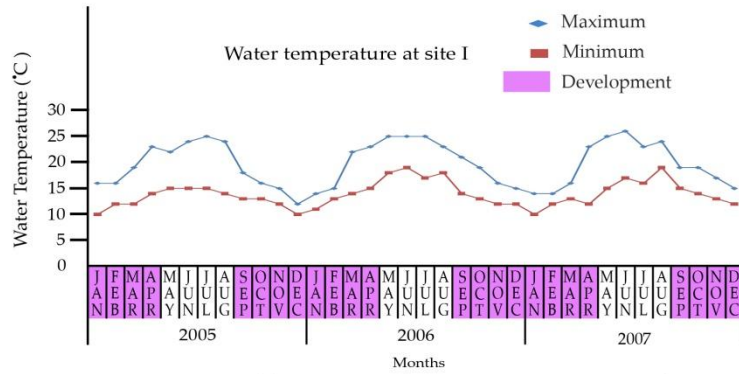


Fig 3.57: Monthly variation of Water temperature at study site I

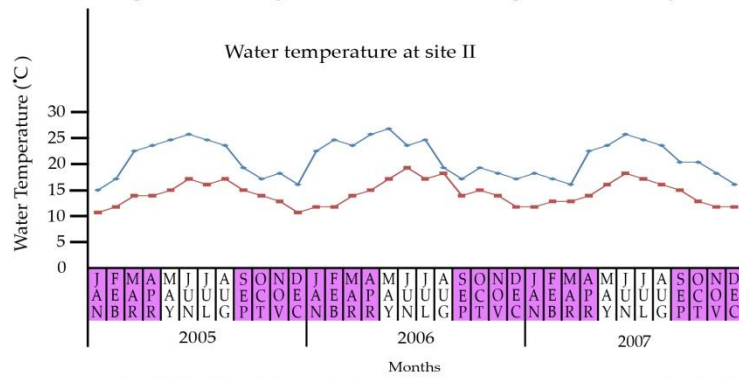


Fig 3.58: Monthly variation of Water temperature at study site II

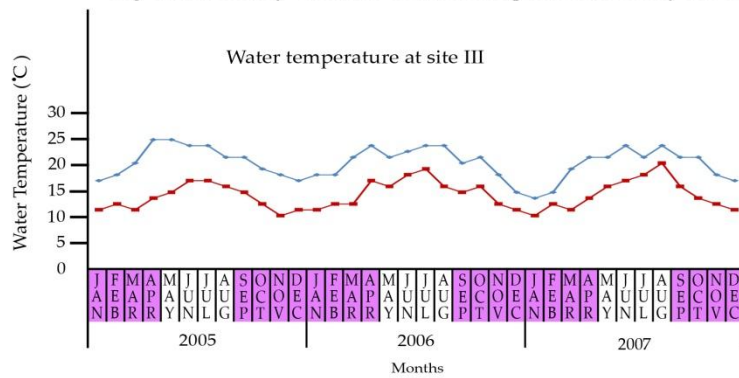


Fig 3.59: Monthly variation of Water temperature at study site III

Chapter IV

Oral structure of the tadpoles and
the food and feeding habits of both
the tadpoles and adults of *Rana alticola*

INTRODUCTION

The feeding strategies of amphibians include their choice of prey and the ways they locate, capture and ingest the prey. Amphibians are generally considered to be feeding opportunists with their diets reflecting the availability of food of appropriate size. Herbivory is characteristic of anuran larvae, but it may occur in other amphibians like the aquatic salamanders of the genus *Siren*. The limited information on amphibian diets indicates that all adult amphibians are carnivores; most feed principally on insects, although many species eat a wide variety of invertebrates. Research has focused on evidence of food selection from several approaches; stomach content analysis (Jenssen 1967); comparative aspects of buccal anatomy related to feeding (Viertel 1985; Wassersug 1980); comparative mechanical restrictions on feeding behavior based on anatomy and limited experimentation (Wassersug and Hoff 1979); feeding dynamics of a small number of species in the laboratory (Seale 1980); and relationships between feeding modes and microhabitats used, oral morphology, diet, and phylogenetic groups (Altig and Johnston 1989; Diaz-Paniagua 1985; Heyer 1973; Inger 1986). Most tadpoles feed by producing currents that carry particles into the buccal cavity and across food entrapment surfaces. A tadpole opens its mouth, depresses the floor of the buccal cavity to draw in water, closes the mouth, and then elevates the floor of the buccal cavity to pump water across food entrapment surfaces and out through the spiracle (De Jongh 1968; Gradwell 1972a, b; Kenny 1969a, b; Severtsov 1969; Wassersug 1972). Tadpoles occur in countless aquatic habitats, feed at many sites (benthic, midwater, and surface) throughout the water column, and have characteristic morphologies and behaviors.

The teeth of frogs function primarily to grasp prey, or to position it for swallowing. The oral armature of the larvae differs from that of the adults, as they differ in feeding habits. The ontogeny of the labial teeth row structure of anuran tadpoles inhabiting temperate regions has been studied by several workers (Taylor 1942, Zweifel 1964, Altig 1970, Lee 1976, Webb and Korky 1977, Hero 1990 and Davies 1992). Rao (1914), Lobo (1961), Chari (1962), Daniel (1975), Inger *et al.*, (1984) and Sekar (1990) have given brief notes on the mouth parts of Indian amphibians. Agarwal and Niazi (1980), and Dutta and Mohanty-Hejmadi (1984) have reported the ontogeny of the teeth row structure in *Hoplobatrachus tigerinus*. James *et al.*, (2000) studied the development of the labial teeth row structure in *Rana curtipes* tadpoles.

Understanding morphology, especially that of the oral disc is crucial to comprehending the feeding ecology of tadpoles. The functional morphology of the oral apparatus of tadpoles is largely unknown at present. Hence, little is known about how the mouthparts work. The intestine is one organ that is remodeled during metamorphosis. The morphological changes that take place during intestinal remodeling are more drastic than those in the liver and brain. The intestinal epithelium is a complex structure that provides an enormous luminal surface area for efficient food processing and absorption, the primary function of the organ (Glass, 1968; Segal and Petras, 1992).

Metamorphosis in anurans involves the most comprehensive and most dramatic transformations of all major living chordate groups. The postembryonic process systematically transforms most, if not all, organs of a tadpole to their adult forms. In addition, it also brings about the development of organs that only function

in the adult frog. There are three major types of changes that take place during metamorphosis. The first is the complete resorption of tadpole-specific organs such as the tail. On the other extreme, frog-specific organs like the hind limb develop de novo from undifferentiated stem cells in a process that involves first the proliferation of stem cells and subsequent cell differentiation and tissue morphogenesis. The last major type of transformation is the partial but profound remodeling of the existing organs like liver and intestine into their adult forms. 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.

REVIEW OF LITERATURE

The morphology and feeding habits of tadpoles have been described in numerous species of anurans. These studies usually focus mainly on morphology or ecology. Thus, studies by Savage (1955), Satel and Wassersug (1981), Ruibal and Thomas (1988), Echeverria (1992 a, b, 1997 a, b, 1998), Iordansky (1992), among others, provide information on morphological and anatomical features, whereas authors such as Kamat (1962), Inger (1986), Lajmanovich (1994), Lajmanovich and Fernandez (1995), and Lavilla (1983) supply ecological analysis. Foraging behaviour is one of the most important components of reproductive fitness (Nishimura 1999). Therefore, the remarkable ability of most group-living organisms to distribute themselves precisely among feeding sites in proportion to habitat profitability is not surprising (Godin and Keenleyside 1984; Talbot and Kramer 1986).

Tadpoles also interact with other types of herbivores. A variety of adults and larvae of herbivorous, aquatic insects, crustaceans, and zooplankton co-occur with tadpoles and use similar resources. Few studies have examined either the impact of tadpoles or other aquatic herbivores on their shared resources or possible interphyletic competitive interactions involving tadpoles. Alford (1989) demonstrated experimentally that tadpole densities affected the growth rates of larval newts, which prey on zooplankton, and suggested that this was probably an indirect effect of competition between tadpoles and zooplankton. Morin *et al.*, (1988) demonstrated that interphyletic competition can influence the growth of tadpoles. Bronmark *et al.*, (1991) found that larval *Rana temporaria* negatively affected the

growth of snails, while snails apparently facilitated the growth of *Rana*, possibly by altering the composition of the algal assemblage.

Mouthparts: The structure and adaptive type of mouthparts provide a very useful characteristic feature to several workers (Altig, 1970; Altig and Pace, 1974; Lee, 1976) for their taxonomic work. Orton (1953 and 1957) based his classification up to frog families on the mouth parts of tadpoles. The mouth parts of anuran tadpoles differ from species to species (Noble, 1954; Daniel, 1975) also their structure depends on their habitat. The structure of tadpole mouth parts of different anuran species has been studied extensively by different workers (Kirtisinghe, 1957; Dutta and Mohanty-Hejmadi, 1984; Gupta and Khare, 1987; Altig and Thibaudeau, 1988; Warkentin, 1999; Hall *et al.*, 2002). The mouth of *R.maximus* is characterized of pond-type of tadpoles ordered laterally and ventrally by one or two rows of small papillae (Khongwir *et al.*, 2003). According to Smith (1924a) and Noble (1954) tadpoles living in swift waters often have larger lips and greater number of teeth rows as compared to those living in stagnant waters.

Das (1994) described in detail the internal oral morphology of some anurans using SEM and correlated these features with the feeding habits of the tadpoles. The oral papillae (submarginal and marginal) whose distribution and density vary greatly in different tadpoles are believed to serve as chemosensory and tactile receptors (McDairmid and Altig 1999). They are also considered to help in the control of water flow, attachment to substrates, alter the shape of oral disc during feeding, and manipulate food and substrate particles. The labial teeth also show wide variations in their occurrence and morphology with respect to shape, size and keratinization. This may be absent in some species. In others, these may be blunt, pointed and with or

without cusps. A functional difference in the morphology of labial teeth is unknown. Probably they serve as broom for current generation, for breaking up mucilaginous layers, sieving particle, combing strands into alignment of easier cutting, piercing plant cells, rasping food particles from a substrate, attaching to a substrate, and trapping food. Likewise, the jaw sheaths also show variations across taxa. The functional aspect of jaw sheath is not clear. Probably they serve as gouging, biting and/or scraping structures. However, their striking differences across taxa indicate innumerable variety of performance abilities.

Food Items: One of the most important evolutionary innovations of anuran amphibians is the derived larval stage, commonly known as the tadpole. In contrast to the other two amphibian orders (i.e, salamanders and caecilians), frog larvae are usually filter-feeding omnivores. They are able to feed both on the phytoplanktonic community by means of filtration, and on a large variety of substrates (including algae, macrophytes and carrion) by rasping, scraping and chopping with their jaw sheaths and labial teeth (Seale and Wassersug, 1979; Seale, 1982). Their digestive system is adapted to processing vegetable matter, and the major components of their mouthparts are not homologous to the jaw apparatus of the vertebrate bauplan (Altig and McDiarmid, 1999).

Most temperate *Rana* tadpoles are generalistic algae feeders (Seale, 1980). Important food categories include filamentous green algae (Dickman, 1968), epiphytic diatoms (Kupferberg, 1997b), detritus (including decomposed higher plants) and various algae (Jenssen, 1967). Grazing by tadpoles potentially reduces algae biomass (Bronmark *et al.*, 1991). A review shows that, generalizing over all grazers, periphyton removal rate increases with increased grazer biomass (Cattaneo

and Mousseau, 1995). However, the effects of grazing by tadpoles and other epiphytic grazers on the algae community can be very complex. By removing epiphyton growing on filamentous algae or macrophytes, the latter are favored by tadpoles (Kupferberg, 1997b) or invertebrates (Dodds, 1991; Underwood, 1991). Also, algae may benefit from increased nutrient levels caused by grazing tadpoles (Osborne and McLachlan, 1985) or invertebrates (Gresens, 1995).

Most anurans breed in ephemeral ponds and puddles of diverse nature that support the growth and abundance of different species of algae, diatoms and plankton. Consequently, the type of food consumed by the tadpoles depends on the availability of food (type and abundance) in a given water body. A majority of anuran tadpoles are herbivorous or omnivorous while a few of them are carnivorous and even cannibals (Kamat 1962, Costa and Balasubramaniam 1965, Sabnis and Kolhatkar 1977, Sabnis and Kuthe 1980, Sekar 1992).

Most tadpoles are suspension feeders (Wassersug, 1972), and the filter apparatus enables them to trap a wide range of particulate food. Tadpoles lacking keratinized mouthparts filter particles suspended in the water column. Tadpoles with keratinized mouthparts scrape or bite food from the substrate and filter the suspension. Many bottom feeders ingest periphyton, detritus and interstitial organisms from the sediment, and large quantities of indigestible material of different sizes (Seale et al. 1982; Seale and Wassersug 1979; Viertel 1990, 1992). Tadpoles usually are considered highly specialized, filter-feeding herbivores (Duellman and Trueb 1986). Most ingest planktonic materials from the water column, obtain organic materials from pond sediments, or scrape material (i.e., aufwuchs, periphyton) from submerged substrates. Given the diverse and sometimes

bizarre morphology of their feeding structures, the variety of sizes and kinds of food items recorded in their guts, the absence of cellulose for digesting plant materials, the diversity of microhabitats occupied, and lack of basic knowledge of their diets determined from conventional gut content analyses convinces that tadpoles are better thought of as opportunistic omnivores or detritivores. Systematic and comparative evaluations of the food habits of tadpoles are uncommon and generally not very informative because many ingested items pass undamaged through the gut, while other soft-bodied organisms and bacteria are not detected. Such observations have led some authors to suggest that the real sources of food for tadpoles may be dissolved inorganic and organic nutrients, bacteria, and viruses (Heyer 1973; Inger 1986). Items reported from the guts of tadpoles include sand, detritus, viruses, bacteria, small unicellular organisms (protists), algae (diatoms, filamentous green, euglenophytes), plant fragments of assorted sizes, pollen grains, fungi, various kinds of small animals (annelids, cladocerans, copepods, gastrotrichs, insects, nematodes, rotifers, tardigrades, water mites), anuran eggs, and heterospecific and conspecific tadpoles (Costa and Balasubramaniam 1965; Diaz-Paniagua 1985; Harrison 1987; Heyer 1973; Inger 1986; Lajmanovich 1994; Sabnis and Kuthe 1980; Sekar 1992). Tadpoles also are known to feed on fecal material (Steinwascher 1978). Majority of anuran tadpoles are herbivorous or omnivorous.

Tadpoles also interact with other types of herbivores. A variety of adults and larvae of herbivorous, aquatic insects, crustaceans, and zooplankton co-occur with tadpoles and use similar resources. Few studies have examined either the impact of tadpoles or other aquatic herbivores on their shared resources or possible interphyletic competitive interactions involving tadpoles. Alford (1989)

demonstrated experimentally that tadpole densities affected the growth rates of larval newts, which prey on zooplankton, and suggested that this was probably an indirect effect of competition between tadpoles and zooplankton. Morin *et al.*, (1988) demonstrated that interphyletic competition can influence the growth of tadpoles. Bronmark *et al.*, (1991) found that larval *Rana temporaria* negatively affected the growth of snails, while snails apparently facilitated the growth of *Rana*, possibly by altering the composition of the algal assemblage.

Metamorphosed amphibians are in general insectivorous; only a few species grow large enough to take small vertebrate prey (Duellman & Trueb, 1986). For such a species-rich group, it was long thought to be remarkable that all known amphibians are carnivorous as adults (Reeder, 1964).

Intestine: The metamorphosis of a tadpole into a frog is one of nature's most spectacular events. Part of this spectacle includes profound changes in the alimentary tract. During metamorphosis there are both microscopic changes in the intestines at the histological level and macroscopic changes in the overall length. These histological changes are well documented. They occur in both the epithelial and extraepithelial layers (Dauca and Hourdry, 1985; Hourdry and Beaumont, 1985). During metamorphosis, the larval gut lining is degraded (Fox, 1984; Yoshizato, 1989). There is increased production of acid phosphatase by lysosomes along the alimentary tract (Kaltenbach *et al.*, 1981), which helps break down the premetamorphic epithelium. Epithelial cells are autolysed and engulfed by phagocytes. Finally, much of the degenerated tissue is sloughed off into the intestinal lumen. Simultaneously, the adult intestinal epithelium differentiates. Nests of undifferentiated precursor cells divide and produce a new epithelium (Marshall and

Dixon, 1978). The connective tissue and musculature of the gut wall also proliferate greatly (Ishizuya Oka and Shimozawa, 1987b). While all of these histological changes are taking place, the intestine shortens extensively. Before metamorphosis the intestine in common frogs, such as those of the genus *Rana*, can be 10 times or more times the body length of the tadpole (Rugh, 1951). Intestinal shortening begins before the tail is absorbed (Dauca and Hourdry, 1985), and by completion of metamorphosis the absolute length of the gut is usually less than a third its premetamorphic length. Some representative values of the percentage that the gut shortens at metamorphosis are; 58.15% in *Rana temporaria*, 82.2% in *Rana pipiens* (Janes, 1934); 90% in *Alytes obstetricians* (Dauca and Hourdry, 1985); and 84% in *R. catesbeiana* (Carver and Frieden, 1977). The epithelial transition from larval to adult form in the amphibian intestine can be divided into two processes, degeneration of the primary epithelium and development of adult (secondary) epithelium. The degeneration of the larval epithelium begins around the onset of metamorphic climax. When the epithelial transition from the larval to adult form begins, the connective tissue suddenly increases in mitotic activity, cell number, and thickness. The connective tissue at this time consists of various types of cells such as immature mesenchymal cells, fibroblasts, macrophages, and mast cells (Ishizuya-Oka and Shimozawa, 1987b, 1992; Yoshizato, 1989). Accompanying these changes in the connective tissue is the profound remodeling of the interface between the connective tissue and the epithelium. When the primary epithelium begins to degenerate, the basal lamina, which is thin throughout the larval period, becomes thick in the entire region beneath the epithelium and remains thick until the primary

epithelium disappears (Ishizuya-Oka and Shimozawa, 1987a, b; Shi and Ishizuya-Oka, 1996).

The intestine in adult amphibians resembles that in higher vertebrates (Reeder, 1964; McAvoy and Dixon, 1977; Dauca and Hourdry, 1985; Ishizuya-Oka and Shimozawa, 1987a; Shi and Ishizuya-Oka, 1996). It has elaborate connective tissue and muscles. The epithelium also forms multiple circular folds; however, the villi and crypts are absent. Instead, the epithelial cells with numerous microvilli line the luminal surface of the folds with the proliferative cells confined toward the trough and differentiated cells towards the crest, thus generating a cell renewal system along the trough-crest axis similar to that in higher vertebrates (McAvoy and Dixon, 1977; Marshall and Dixon, 1978; Shi and Ishizuya-Oka, 1996). The tadpole intestine on the other hand, has a much longer but simpler structure. It consists of a single layer of columnar epithelium surrounded by thin layers of muscles with little intervening connective tissue (McAvoy and Dixon, 1977; Kordylewski, 1983; Ishizuya-Oka and Shimozawa, 1987a; Shi and Ishizuya-Oka, 1996). There is only a single epithelial fold, the typhlosole, present in the anterior part of the intestine where larval connective tissue is abundant. These structural differences between larval and adult intestines presumably reflect changes in the physiological functions between herbivorous tadpoles and carnivorous frogs. This contrasting morphology of the intestine in tadpoles and frogs together with the relatively simple spatial organization of its different tissues, for example, the epithelium, connective tissue, and muscles, has encouraged extensive investigation of the morphological changes of the organ during metamorphosis. At the gross anatomic level, the long larval small intestine suddenly begins to shorten around the onset of metamorphic climax

and this process continues until the end of metamorphosis (Marshall and Dixon, 1978; Ishizuya-Oka and Shimosawa, 1987a). The region of the small intestine containing the typhlosole remains relatively constant, about one-third of the small intestine, in tadpoles and during early metamorphic climax. After stage 43, the typhlosole is no longer recognizable as the morphogenesis of intestinal folds takes place (Marshall and Dixon, 1978; Ishizuya-Oka and Shimosawa, 1987a). These intestinal folds appear as several circular folds that run longitudinally and are straight along the gut axis, gradually increasing in number and height, and finally being modified into longitudinally zigzagged folds (Shi and Ishizuya-Oka, 1996). The zigzag folds then remain throughout adulthood (McAvoy and Dixon, 1978a, b).

MATERIALS AND METHODS

In order to study the food and feeding habits of *Rana alticola*, different developmental stages of the tadpoles were collected. The adult frogs were collected from Sairang River (Study site I), Herhse stream (Study site II) and Tamdil Lake (Study site III). Soon after collection, the specimens were euthanized in MS-222 and preserved in 4% formaldehyde at the field itself in order to prevent complete digestion of the ingested food particles. After fixation, the materials were brought to the laboratory. The tadpoles were sorted out stage wise according to Gosner (1960), after which they were autopsied for analysis of the gut contents. After dissection, the guts were removed and measured with the help of ruler and dial caliper before flushing with distilled water. The gut contents were then taken in a clean slide and analysis of the gut contents was done under a compound microscope. Photographs of the gut contents were taken with the help of Sony cybershot (5.1 megapixels, DCS-W5) attach binocular (Labomed, CMS-2). Identification of the gut contents of the tadpoles was made following Edmonson (1957). The adult frogs were also dissected and the gut contents were analysed under a stereo-scopic dissecting binocular microscope. Analysis of histological changes of the gut of the tadpoles and adult was carried out with the help of a compound microscope. The oral structures of the selected developing tadpoles were studied using light microscopy, stereo-scopic binocular microscope following the criteria of Altig and McDiarmid (1999), and also with Scanning Electron Microscopy.

For scanning electron microscopy, the samples were fixed in 2.5 % glutaraldehyde solution (prepared in 0.1 M Sodium Cacodylate buffer) for four hours. The pH of fixative and buffer was maintained at 7.4. The samples were then

dehydrated through ascending acetone grades and drying was done in Tetra methyl silane (Dey *et al.*, 1989). A thin conductive coating of gold was applied to the samples using a JFC 1100 (Jeol) ion sputter and the coated samples were examined with the aid of a JSM -6360 (Jeol) scanning electron microscope at an accelerating voltage of 20KV. Working distance was 10 mm and the necessary tilting angles were used to view the frontal portion of the oral structures.

For TEM studies, portion of the small intestine was cut and fixed in Kornovsky's fixative (Prepared in 0.1 M Sodium-Cacodylate buffer) in 0.1 M Cacodylate buffer (pH 7.4) at 4°C for 2 hours and post-fixed with 1% Osmium tetroxide buffered (0.1 M Na-Cacodylate buffer) at 4°C for 1 hour. The samples were then dehydrated through acetone grades (30%, 40%, 50%, 60%, 70%, 80%, 90% and 100 %) and then cleared in propylene oxide. The samples were then infiltrated in a mixture of clearing agent and embedding medium. After infiltration the tissues were embedded in the epoxy resins using beam capsules and blocks were prepared. Ultrathin sections (60 – 90 nm) were cut with the help of an ultra microtome (Ultratome V, LKB), stained with uranyl acetate and lead citrate and were examined with a Transmission electron microscope (JEM 100C x II Jeol) at an accelerating voltage of 80 KV. Semi thin sections (500 nm) were cut using the Ultra-microtome which was floated on double distilled water. The Sections were then lifted up on to a slide containing a drop of distilled water, and kept for drying and stretching in a hot-plate at 50-60°C. The dried section on the slide were then covered with Toulidine Blue Stain and kept on the Hot plate at 60°C for 10-30 Sec. The excess stain was washed in gentle tap water. The slides were dried and viewed under a compound microscope and photographed.

RESULTS

ORAL STRUCTURE OF THE TADPOLE OF *Rana alticola*:

The oral structures of the tadpole of *Rana alticola* was studied with the help of a stereoscopic binocular microscope and scanning electron microscope.

When observed under the stereoscopic binocular microscope, it was revealed that, the oral disk is large, anteroventral, not laterally emarginated, directed ventrally. The keratodont starts to appear from stage 25. The mouthpart at stage 25 is simple with labial tooth row formula (LTRF) which is not consistent; and may vary from 1- 2: (2+2) - (4+4) / 1+1: 3-5. e. g., at stage 25 (Fig 4.1) the oral structure of the tadpole is characterized with one uninterrupted serrations of teeth row and two distinct interrupted rows on the upper labium. The lower labium consists of one interrupted teeth row followed by three uninterrupted teeth row. The LTRF is 1:2+2/1+1:2 (Fig 4.1).

The present study established that *Rana alticola* tadpoles have marginal teeth which make their first appearance at stage 26 (Fig 4.2). The number of tooth rows or keratodont rows increases during ontogeny. The LTRF remains the same from stage 26 (Fig 4.2) through stage 41. However, the LTRF at these stages are not consistent and it may vary from 2: (3+3) - (5+5) / 1+1: 4-7. e. g., at stage 26 (Fig 4.2) the oral structure of the tadpole is characterized with two uninterrupted serrations of teeth row and five distinct interrupted rows on the upper labium. The lower labium consists of one interrupted teeth row followed by seven uninterrupted teeth row. The LTRF is 2:5+5/1+1:7(Fig 4.2).

The oral structure starts to degenerate when the forelimb emerged at stage 42 (Fig 4.3). By the time the tadpole reach stage 43 (Fig 4.4), the labial tooth row has disappeared completely.

Scanning electron microscopic studies of *Rana alticola* tadpoles at stage 26 reveals that, the oral disc is composed of anterior (upper) labium (AL) and posterior (lower) labium (PL). The upper (anterior) labial tooth rows are designated as A1, A2, A3, A4 and A5 and the lower (posterior) labial tooth rows are designated as P1, P2, P3, P4, P5, P6 and P7. There are total twelve keratodonts rows, five anteriorly (A) and seven posteriorly (P). Tooth rows A-1, A-2, P-2, P-3, P-4, P-5, P-6 and P-7 are complete and extend from one commissure to the other, whereas A-3, A-4, A-5 and P-1 are interrupted medially (making the labial tooth row formula 2:3+3/1+1:6). From stage 26 onwards, the tooth row A-1, A-2 and P-1 remains constant. Marginal papillae (MP) is present on the edge of the oral disc with a wide dorsal gap (G), sub marginal papillae (SM) is also present. The upper jaw sheath (UJS) and lower jaw sheath (LJS) is clearly visible (Fig 4.5). Higher magnification reveals that the Upper beak is straight while the lower beak is with a central V-shaped groove with the mouth (M) in between (Fig 4.6). When magnified, the suprarostrodont (Sr) appeared to be serrated and pointed where the length measures about 40 μm while the breadth is 11 μm (Fig 4.7), while the Infrarostrodont (Ir) measures about 60 μm in length and 25 μm to 30 μm in breadth and appeared serrated and blunt (Fig 4.8).

Margins of the labia are defined by papillae, this marginal papillae has a wide dorsal gap (G) which is about 2mm to 3mm in length depending on the size of the tadpole; the gap covers almost whole upper lip, as wide as length of first upper tooth row (Fig 4.9). In *Rana alticola*, the tooth rows are uniserial and the labial tooth is

keratinized and each keratinized labial tooth is derived from cells in the base of the tooth ridge and consists of three indistinct regions; a distal head (C) with around 20 terminal cusps, an intermediate body known as the neck (N) and a basal hollow sheath known as the base (B) (Fig 4.10). The cusp measure 60 μm to 66 μm in length. Submarginal papillae are positioned in lateral parts of upper and lower labia, both the marginal and submarginal papillae has a rounded tip and the papillae are closely spaced, short and numerous in number (Fig 4. 11 and 4.12). The papillae measures 70 μm to 80 μm in length, and 35 μm to 40 μm (at the broadest part) in breadth. The space between each marginal papillae (MP) on the anterior labium range from 31 μm to 37 μm (Fig 4.13) and the space between each marginal papillae (MP) on the posterior labium range from 12 μm to 25 μm (Fig 4.14). At some point, bifid papillae (P) are also seen in the tadpole of *Rana alticola* (Fig 4.15). At the later part of stage 41, the rostrodont (R) starts to degenerate (Fig. 4.16). Scanning electron micrograph shows that at stage 42 when the forelimbs have emerged, the labial teeth start to degenerate (Fig 4.17). Also at this stage, the papillae continue to degenerate (Fig 4.18) and at stage 43, the labial teeth have shed completely (Fig 4.19) with only a few papillae still persisting in small cluster at the angle of jaw (Fig 4.20).

Food items of *Rana alticola*

Tadpoles: Qualitative analysis of gut contents of the tadpoles and adults of *Rana alticola* was done and observations revealed that tadpoles started feeding from stage 25 onwards. The gut contents of the tadpoles mainly include phytoplankton like Euglenophyceae, Cyanophyceae, Chlorophyceae and Bacillariophyceae and zooplankton like Rotifera, Rhizopoda, Cladocera and Copepoda.

Stage 25: The gut contents of stage 25 consists of phytoplankton which includes Euglenophyceae like *Euglena*, Cyanophyceae like *Oscillatoria* and *Anabaena*, Chlorophyceae like *Spirogyra*, *Cosmarium*, *Staurastrum*, *Closterium*, *Scenedesmus*, *Pediastrum*, *Ulothrix*, *Zynema* and *Selenastrum*, and Bacillariophyceae like *Navicula*, *Diatoma*, *Stauroneis* and *Tabellaria*. (Table 4.1; Fig 4.21)

Zooplankton includes Rotifera like *Brachionus patulus*, *Colurella obtusa*, *Philodina sp.*, *Polyarthra sp.*, and *Trichotria tetractis*, Rhizopoda like *Arcella megastoma*, and *Arcella vulgaris*, Cladocera like *Alona sp.* and *Bosmina*, and Copepoda like *Nauplius larva*. (Table 4.2; Fig 4.22)

Stages 26-30: The gut contents of stages 26-30 consists of phytoplankton which includes Euglenophyceae like *Euglena*, Cyanophyceae like *Oscillatoria*, *Anabaena* and *Nostoc* Chlorophyceae like *Cosmarium*, *Staurastrum*, *Closterium*, *Scenedesmus*, *Pediastrum*, *Ulothrix*, *Zynema* and *Selenastrum*, and Bacillariophyceae like *Navicula*, *Diatoma*, *Stauroneis* and *Tabellaria*. (Table 4.1; Fig 4.21)

Zooplankton which includes Rotifera like *Brachionus patulus*, *Colurella obtusa*, *Lecane thlera*, *Philodina sp.*, and *Polyarthra sp.*, Rhizopoda like *Arcella megastoma*, *Arcella vulgaris* and *Euglypha sp.* Cladocera like *Alona sp.* and *Bosmina*, and Copepoda like *Nauplius larva*, *Calanoid sp.* and *Cyclops*. (Table 4.2; Fig 4.22)

Stages 31-41: The gut contents of stages 31-41 consists of phytoplankton which includes Cyanophyceae like *Anabaena* and *Nostoc*. Chlorophyceae like *Closterium* and *Pediastrum* and Bacillariophyceae like *Diatoma*, *Stauroneis* and *Tabellaria* (Table 4.1; Fig 4.21)

Zooplankton which includes Rotifera like *Brachionus patulus*, *Colurella obtusa*, *Lecane sinuate*, *Lecane thlera*, *Philodina sp.*, *Polyarthra sp.* and *Trichotria tetractis* Rhizopoda like *Arcella megastoma*, *Arcella vulgaris* and *Euglypha sp.* Cladocera like *Alona sp.* and *Bosmina*, and Copepoda like *Nauplius larva*, *Calanoid sp.* and *Cyclops* (Table 4.2; Fig 4.22). Cannibalism was observed in the tadpole of *Rana alticola* during the time of food shortage in the laboratory condition.

Stages 42 – 46: From stage 42 onwards which coincide with the emergence of the forelimbs, the tadpoles cease to feed. From stage 46 onwards, the froglet start to feed on small invertebrates.

Adults: Observation of the gut contents of the adult frog of *Rana alticola* revealed that the diet of the adult frog mainly includes insects. Apart from insects, the diet also includes annelids, crustaceans, fingerlings and some plant materials. Insects include the order Isoptera (e.g. Termites), Lepidoptera (e.g. butterfly), Hymenoptera (e.g. ants), Coleoptera (e.g. beetle), Orthoptera (e.g. grasshopper), Hemiptera and Dipteran flies. On some instances plastic materials, sand particles and pieces of small stones were also found in the gut of the adults (Fig 4.23).

Histological and Ultra structural study of the intestine of the tadpoles and adult of *Rana alticola*:

The intestine is one organ that is remodeled during metamorphosis. The morphological changes that take place during intestinal remodeling are more drastic and the intestinal epithelium is a complex structure that provides an enormous luminal surface area for efficient food processing and absorption, the primary function of the organ. The tadpole intestine has a long and simple structure. It

consists of layers of columnar epithelium surrounded by thin layers of muscles with little intervening connective tissue.

Stage 25: The length of the intestine at this stage range from 30 mm to 105 mm (mean 66.4 mm \pm 24.54 mm) as compared to the total length of the tadpole which range from 10 mm to 31.71 mm (mean 17.39 mm \pm 6.9 mm). There is significant positive correlation between the tadpole length and the gut length where $r = 0.94$; $p < 0.5$ (Table 4.3). At this stage, the tadpole start to feed, and the intestine was observed to be a simple tubular structure consisting of larval epithelial cells (Fig 4. 24). Ultra structural study revealed that at this stage the brush border appears to be long and compact and the nucleus is clearly visible (Fig 4.30).

Stage 32: The length of the intestine at this stage range from 212 mm to 289 mm (mean 250.3 mm \pm 24.25 mm) as compared to the total length of the tadpole which range from 51.5 mm to 65.74 mm (mean 60.08 mm \pm 4.84 mm). There is significant positive correlation between the tadpole length and the gut length where $r = 0.897$; $p < 0.5$ (Table 4.3). The tadpole intestine is longer as compared to stage 25.

Stage 38: The length of the intestine at this stage range from 180 mm to 510 mm (mean 358.2 mm \pm 115.87 mm) as compared to the total length of the tadpole which range from 60.36 mm to 95.14 mm (mean 78.89 mm \pm 11.41 mm). There is significant positive correlation between the tadpole length and the gut length where $r = 0.903$; $p < 0.5$ (Table 4.3). The intestine continues to grow with the growth in size of the tadpole. Ultra structural study revealed that at this stage the brush border appears to be long and compact and the nucleus is clearly visible (Fig 4.30).

Stage 41: The length of the intestine at this stage range from 226 mm to 486 mm (mean 390.7 mm \pm 75.8 mm) as compared to the total length of the tadpole which

range from 52.9 mm to 91.54 mm (mean 79.4 mm \pm 12.15 mm). There is significant positive correlation between the tadpole length and the gut length where $r = 0.82$; $p < 0.5$ (Table 4.3). The tadpole intestine consists of layers of columnar epithelium cells surrounded by thin layers of muscles with little intervening connective tissues (CT) (Fig 4. 25).

Stage 42: The length of the intestine at this stage range from 97 mm to 135 mm (mean 122.5 mm \pm 13.35 mm) as compared to the total length of the tadpole which range from 64.8 mm to 92.2 mm (mean 77.19 mm \pm 9.2mm). There is significant positive correlation between the tadpole length and the gut length where $r = 0.72$; $p < 0.5$ (Table 4.3). At this stage, the forelimbs have emerged and the intestine starts to shorten from this stage. The connective tissue shows increase in thickness and the epithelial folds starts to form at this stage (Fig 4. 26). Ultra structural study revealed that at this stage the brush border appears to be long but less compact as compared to the previous stages. (Fig 4.30)

Stage 45: The length of the intestine at this stage range from 59 mm to 84 mm (mean 68.3 mm \pm 7.41 mm) as compared to the total length of the tadpole which range from 37 mm to 61 mm (mean 46.71 mm \pm 7.07 mm). There is significant positive correlation between the tadpole length and the gut length where $r = 0.89$; $p < 0.5$ (Table 4.3). The length of the intestine has greatly reduced at this stage. When the epithelial transition from the larval to adult form begins, the connective tissue (CT) suddenly increases in mitotic activity, cell number, and thickness and the epithelial folds become more prominent (Fig 4. 27).

Stage 46: The length of the intestine at this stage range from 36 mm to 64 mm (mean 50.6 mm \pm 7.71 mm) as compared to the total length of the tadpole which

range from 17 mm to 39.1 mm (mean 27.07 mm \pm 6.5 mm). There is significant positive correlation between the tadpole length and the gut length where $r = 0.74$; $p < 0.5$ (Table 4.3). There is profound remodeling of the interface between the connective tissue (CT) and the epithelium. When the primary epithelium begins to degenerate, the basal lamina, which is thin throughout the larval period, becomes thick until the primary epithelium disappears (Fig. 4. 28). The intestine at this stage resembles that of the adult intestine. Ultra structural study revealed that at this stage the brush border appears to be shorter and less compact (Fig 4.30).

Adults: The intestine in adult amphibians resembles that in higher vertebrates. It has elaborate connective tissue (CT) and muscles (M). The primary epithelium (PE) degenerates and the secondary epithelium (SE) are formed. The epithelium also forms multiple circular folds; however, the villi and crypts are absent. Instead, the epithelial cells with numerous microvilli line the luminal surface of the folds with the proliferative cells confined toward the trough and differentiated cells towards the crest, thus generating a cell renewal system along the trough-crest axis similar to that in higher vertebrates. These intestinal folds (Fo) appear as several circular folds that run longitudinally and are straight along the gut axis, gradually increasing in number and height, and finally being modified into longitudinally zigzagged folds. The zigzag folds then remain throughout adulthood (Fig 4.29).

DISCUSSION

Rana alticola tadpole belongs to the lotic-benthic ecomorphological guild of Altig & Johnston (1989) where the tadpole has keratinized mouthparts with which they rasp food from submerged surfaces. The present study established that *Rana alticola* tadpoles have marginal teeth which make their first appearance at stage 26 which is also seen in *Rana curtipes* tadpoles where the marginal teeth make their first appearance in the pre limb stage. Similar findings have been reported in *R. pustolusa* (Taylor 1942), *R. tarahumarae* (Zweifel 1955) and *R. macroglossa* (Volpe and Harvey 1958). But none have reported the presence of marginal teeth in a tropical anuran. In *Rana alticola*, the number of tooth rows or keratodont rows increases during ontogeny which has also been reported by James *et al.*, (2000) in *Rana curtipes*, where the number of teeth rows changed with growth. There are variations in the development of labial teeth row structure in tropical anuran where the number of teeth rows changes with the stage of development, and each stage there are individual variations. In *Rana alticola*, LTRF remains the same from stage 26 to 41 and before the onset of metamorphosis, labial teeth begin to shed and disappear with the completion of metamorphosis. James *et al.*, (2000) and Dutta and Mohanty-Helmadi (1984) reported a similar pattern in *Rana curtipes* and *Rana tigerina* (now *Hoplobatrachus*) respectively. Slight differences occur in LTRF reported in the previous descriptions of *Rana alticola* tadpoles. The highest number of rows in the present study is 2:5+5/1+1:7 which is more or less similar to the one reported by Annandale (1912) and Sahu & Khare (1980) where the LRTF are 2:5+5/1+1:8 and 2:5+5/1+1:6 respectively from the northeastern Indian tadpoles. Inthara *et al.*, (2005) also reported the LRTF of *Rana alticola* to be 2:5+5/1+1:6

from Thailand but did not mention the stage. However, Grosjean *et al.*, (2003) reported the highest number of rows to be 2:7+7/1+1:8 at stage 38 from Phang Nga Province, Thailand. Ontogenetic variation is common in species having high number of keratodonts rows such as North American pelobatids (Bresler & Bragg, 1954; Gosner & Black, 1954; Bragg & Hayes, 1963; Bragg *et al.*, 1963; Hampton & Volpe, 1963), tadpoles of *Hoplobatrachus*, a ranid genus (Lamotte & Zuber-Vogeli, 1954; Dutta & Mohanty-Hejmadi, 1984) and *Leptobrachium (Vibrissaphora) echinatum*, a megophryid species (Grosjean, 2001). On the basis of present study, it was observed that the number of teeth rows changed during the development and metamorphosis of the tadpoles of *Rana alticola*.

Observations during the present study shows that the tadpoles of *Rana alticola* start feeding from stage 25 onwards. It was found that the tadpoles feed on both phytoplankton and zooplankton. Tadpoles soon stop feeding at stage 42 and after metamorphosis the froglet start feeding on a carnivorous diet. Observations on the gut contents of *Rana alticola* revealed the presence of Euglenophyceae, Cyanophyceae, Chlorophyceae and Bacillariophyceae. Knowledge of food and feeding behavior of the tadpoles is very essential as early part of the life history of amphibian is dependent on the availability of the food items in the natural habitat. Qualitative analysis of food spectrum of five species (*Bufo malanostictus*, *Rhacophorus maximus*, *Amolops afghanus*, *Rana danieli* and *Euphlyctis cyanophlyctis*) of anuran tadpoles from Arunachal Pradesh, India by Sinha *et al.*, (2001) recorded the presence of Diatoms and Chlorophyceae in all the five species which was also seen in the case of *Rana alticola*. The gut content analysis of tadpoles gives a clear idea of the food items present in their habitat. Earlier record of

study on food habit of tadpoles in the north eastern India is that of Sahu and Khare (1988) who studied food and feeding habits of *Rana alticola* tadpoles in Shillong, where they observed that in the early part of life history, tadpoles are herbivorous which later changes to carnivorous in the post metamorphic stages. During the present study, it was observed that cannibalism was seen in the tadpole of *Rana alticola* during the time of food shortage in the laboratory condition. Similarly, tadpoles of *Scaphiopus couchii* also exhibited cannibalism and cannibalistic tadpoles may reach metamorphosis more quickly and at larger sizes reducing the risk of desiccation as pools dry up, and ultimately increasing their fitness (Dayton and Wapo, 2002). It may be suggested that anuran larvae may be important predators in aquatic communities. Several studies have documented cannibalistic and interspecific predation by anuran larvae on eggs, tadpoles, and newly metamorphosed amphibians (Polis and Meyers, 1985; Petranka and Kennedy, 1999).

Tadpoles of *Rana alticola* soon stop feeding at stage 42 and after metamorphosis the froglet start feeding on a carnivorous diet. As an individual grows larger, the morphological changes in its feeding apparatus, including the number of teeth and gape size, allow a wider selection of prey items which was also reported by earlier workers (Bell, 1975; Labanick, 1976; Toft, 1980; Christian, 1982). The absence of keratodonts excludes surface-rasping in some species and, conversely, the presence of robust rostrodonts and multiple tooth rows signals food removal by surface rasping (Candioti, 2004).

Observation of the gut contents of the adult frog of *Rana alticola* revealed that the diet of the adult frog mainly includes insects. Apart from insects, the diet also includes annelids, crustaceans, fingerlings and some plant materials. Insects

include the order Isoptera (e.g. Termites), Lepidoptera (e.g. butterfly), Hymenoptera (e.g. ants), Coleoptera (e.g. beetle), Orthoptera (e.g. grasshopper), Hemiptera and Dipteran flies. On some instances plastic materials, sand particles and pieces of small stones were also found in the gut of the adults (Fig. 4.23). The present findings indicates high percentage of terrestrial food items in *Rana alticola* which agree with the findings of earlier study on stream breeding ranids like *Rana longicrus*, (Kam *et al.*, 1995); *Rana swinhoana*, (Kam *et al.*, 1998). Similar observations have also been reported by Pope and Matthews (2002) that adults of *Rana muscosa* prey on a variety of organisms, including aquatic and terrestrial invertebrates. Erfteimeijer and Boeadt (1991) reported that *Rana chalconota* feeds on terrestrial as well as aquatic insects. Coleopterans, Dipterans and Hymenopterans are also known to be the major foods of *Rana arvalis* and *Rana dalmatina* (Aszalós *et al.*, 2005). The beetles are also basic food for other populations of *Rana arvalis* (Itäimes 1982, Török & Csörgő 1992) and *Rana dalmatina* (Török & Csörgő 1992), most probably due to the abundance of this food and the wide range of environments where it can be found. Other important prey animals are the spiders and the caterpillars.

In *Rana alticola*, it was found that the tadpole starts to feed at stage 25 where the intestine was observed to be a simple tubular structure consisting of larval epithelial cells, and as the tadpole continues to grow the tadpole intestine starts to form multiple circular folds. The adult *Rana alticola* intestinal folds appear as several circular folds that run longitudinally and are straight along the gut axis, gradually increasing in number and height, and finally being modified into longitudinally zigzagged folds. The zigzag folds then remain throughout adulthood. These structural differences between larval and adult intestines presumably reflect

changes in the physiological functions between herbivorous tadpoles and carnivorous frogs. At the gross anatomic level, the long larval small intestine suddenly begins to shorten around the onset of metamorphic climax and this process continues until the end of metamorphosis (Marshall and Dixon, 1978; Ishizuya-Oka and Shimozawa, 1987a).

In *Rana alticola*, as the tadpoles continue to grow, so does the intestine. And as the tadpole regresses from stage 42 onwards, the intestine also begins to shorten. Before metamorphosis the intestine in common frogs, such as those of the genus *Rana*, can be 10 times or more times the body length of the tadpole (Rugh, 1951). Intestinal shortening begins before the tail is absorbed (Dauca and Hourdry, 1985), and by completion of metamorphosis the absolute length of the gut is usually less than third its premetamorphic length. Some representative values of the percentage that the gut shortens at metamorphosis are; 58.15% in *Rana temporaria*, 82.2% in *Rana pipiens* (Janes, 1934); 90% in *Alytes obstetricians* (Dauca and Hourdry, 1985); and 84% in *R. catesbeiana* (Carver and Frieden, 1977). Ultra structural study of the intestine of *Rana alticola* tadpole revealed that at stage 25 the microvilli composing brush border appears to be long and compact but decreases in number and height around the onset of metamorphic climax as also reported by Bonneville (1963); Bonneville and Wienstock (1970) and Hourdry and Dauca (1977).

Table 4.1: Food items (Phytoplankton) of *Rana alticola*

| Food Items | | Stage 25 | Hindlimb bud development Stage 26 - 30 | Toe Differentiation Stage 31 – 41 |
|--------------------------|---------------------|----------|---|--------------------------------------|
| Euglenophyceae | <i>Euglena</i> | + | + | - |
| Cyanophyceae | <i>Oscillatoria</i> | + | + | - |
| | <i>Anabaena</i> | + | + | + |
| | <i>Nostoc</i> | - | + | + |
| Chlorophyceae | <i>Spirogyra</i> | + | - | - |
| | <i>Cosmarium</i> | + | + | - |
| | <i>Staurastrum</i> | + | + | - |
| | <i>Closterium</i> | + | + | + |
| | <i>Scenedesmus</i> | + | + | - |
| | <i>Pediastrum</i> | + | + | + |
| | <i>Ulothrix</i> | + | + | - |
| | <i>Zynema</i> | + | + | - |
| | <i>Selenastrum</i> | + | + | - |
| Bacillariophyceae | <i>Navicula</i> | + | + | - |
| | <i>Diatoma</i> | + | + | + |
| | <i>Stauroneis</i> | + | + | + |
| | <i>Tabellaria</i> | + | + | + |

+ = Occurrence - = Non occurrence

Table 4.2: Food items (Zooplankton) of *Rana alticola*

| Food Items | | Stage 25 | Hindlimb bud development Stage 26 - 30 | Toe Differentiation Stage 31 – 41 |
|------------------|-----------------------------|----------|---|--------------------------------------|
| ROTIFERA | <i>Brachionus patulus</i> | + | + | + |
| | <i>Colurella obtusa</i> | + | + | + |
| | <i>Lecane sinuate</i> | - | - | + |
| | <i>Lecane thlera</i> | - | + | + |
| | <i>Philodina sp.</i> | + | + | + |
| | <i>Polyarthra sp.</i> | + | + | + |
| | <i>Trichotria tetractis</i> | + | - | + |
| | <i>Arcella megastoma</i> | + | + | + |
| RHIZOPODA | <i>Arcella vulgaris</i> | + | + | + |
| | <i>Euglypha sp.</i> | - | + | + |
| | <i>Alona sp.</i> | + | + | + |
| CLADOCERA | <i>Bosmina</i> | + | + | + |
| | <i>Nauplius larva</i> | + | + | + |
| COPEPODA | <i>Calanoid sp.</i> | - | + | + |
| | <i>Cyclops</i> | - | + | + |

+ = Occurrence - = Non occurrence

Table 4.3: Total Length and Gut Length of *Rana alticola*.

| Sl. No. | Stage 25 | | Stage 32 | | Stage 38 | |
|---------------|-----------------------|-----------------|-----------------------|-----------------|-----------------------|-----------------|
| | Total length (mm) | Gut Length (mm) | Total length (mm) | Gut Length (mm) | Total length (mm) | Gut Length (mm) |
| 1 | 13.88 | 52 | 65.74 | 270 | 84.76 | 423 |
| 2 | 10 | 30 | 60.6 | 239 | 60.36 | 183 |
| 3 | 22 | 78 | 62.82 | 245 | 77 | 386 |
| 4 | 31.71 | 105 | 64 | 278 | 82.9 | 480 |
| 5 | 21.27 | 92 | 62 | 265 | 90.7 | 370 |
| 6 | 16 | 70 | 56 | 230 | 71 | 290 |
| 7 | 11 | 45 | 51.5 | 212 | 95.14 | 510 |
| 8 | 22 | 81 | 54.07 | 232 | 78.24 | 310 |
| 9 | 10 | 37 | 59.02 | 243 | 62.82 | 180 |
| 10 | 16 | 74 | 65 | 289 | 86 | 450 |
| Mean ±(SE) | 17.39 ± 6.90 | 66.4 ± 24.54 | 60.08 ± 4.84 | 250.3 ± 24.25 | 78.89 ± 11.41 | 358.2±115.87 |
| Pearson corr. | 0.94 | | 0.897 | | 0.903 | |
| P. Value | 5.76x10 ⁻⁵ | | 4.26x10 ⁻⁴ | | 3.37x10 ⁻⁴ | |

| Sl. No. | Stage 41 | | Stage 42 | | Stage 45 | | Stage 46 | |
|---------------|-------------------|-----------------|-------------------|-----------------|-----------------------|-----------------|-------------------|-----------------|
| | Total length (mm) | Gut Length (mm) | Total length (mm) | Gut Length (mm) | Total length (mm) | Gut Length (mm) | Total length (mm) | Gut Length (mm) |
| 1 | 73.46 | 369 | 69.4 | 102 | 37 | 61 | 23 | 52 |
| 2 | 52.9 | 226 | 72.5 | 121 | 45 | 71 | 39.1 | 64 |
| 3 | 91.54 | 398 | 92.2 | 134 | 40.5 | 59 | 32 | 57 |
| 4 | 90 | 383 | 85.6 | 129 | 43 | 67 | 31.8 | 52 |
| 5 | 91.28 | 440 | 86 | 135 | 61 | 84 | 17 | 36 |
| 6 | 79.6 | 450 | 70.02 | 129 | 41.58 | 60 | 20.4 | 50 |
| 7 | 87.46 | 486 | 64.8 | 97 | 53 | 68 | 24.4 | 56 |
| 8 | 82 | 456 | 83.5 | 122 | 48 | 71 | 25 | 46 |
| 9 | 77 | 375 | 78.45 | 135 | 52 | 73 | 27 | 44 |
| 10 | 68.8 | 324 | 69.45 | 121 | 46 | 69 | 31 | 49 |
| Mean ±(SE) | 79.4± 12.15 | 390.7± 75.8 | 77.19± 9.2 | 122.5± 13.35 | 46.7± 17.07 | 68.3± 7.41 | 27.07± 6.5 | 50.6± 7.71 |
| Pearson corr. | 0.82 | | 0.72 | | 0.89 | | 0.74 | |
| P. Value | 0.0038 | | 0.02 | | 4.74x10 ⁻⁴ | | 0.014 | |



Fig 4.1: Oral structure of stage 25 of *Rana alticola* LTRF 1:2+2/1+1:2

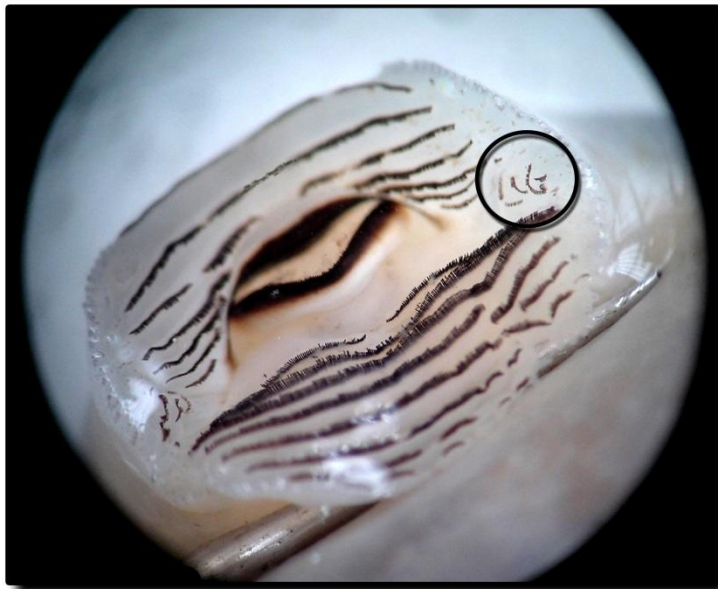


Fig 4.2: Oral structure of stage 26 of *Rana alticola* showing marginal teeth
LTRF 2:5+5/1+1:7



Fig 4.3: Oral structure of stage 42 of *Rana alticola*



Fig 4.4: Oral structure of stage 43 of *Rana alticola*

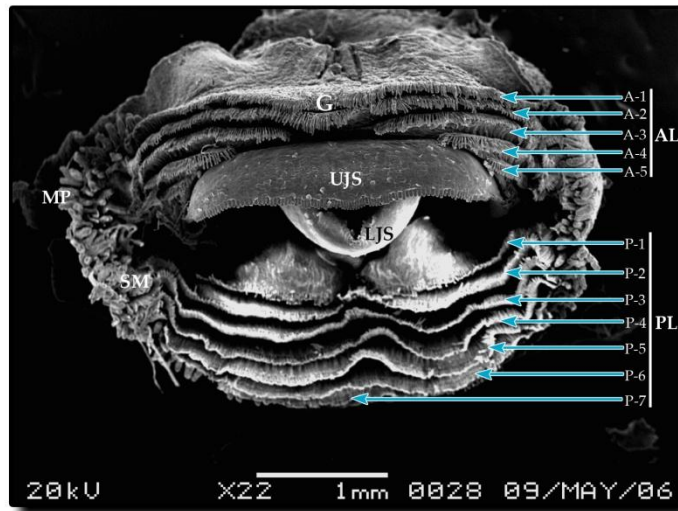


Fig 4.5: Scanning electron micrograph of oral structure of stage 26 of *Rana alticola*. AL=anterior labium; PL=posterior labium; A-1, A-2, A-3, A-4, A-5= 1st through 5th anterior tooth rows; P-1, P-2, P-3, P-4, P-5, P-6, P-7= 1st through 7th posterior tooth rows; MP=marginal papillae; SM=submarginal papillae; G=dorsal gap in marginal papillae; UJS=upper jaw sheath; LJS=lower jaw sheath

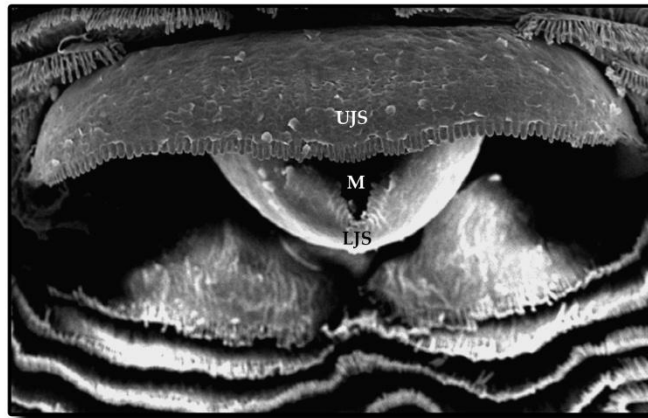


Fig 4.6: Scanning electron micrograph of the mouth (M) of stage 26 of *Rana alticola* with close up view of UJS=upper jaw sheath and LJS=lower jaw sheath

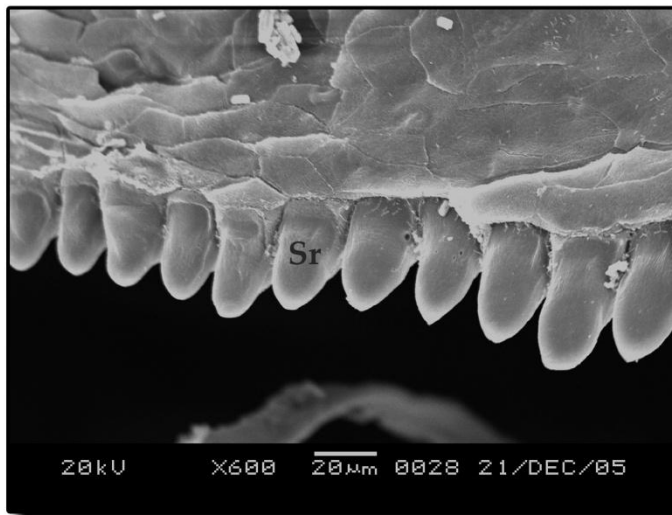


Fig 4.7: Scanning electron micrograph of the suprarostrodont (Sr) of stage 26 of *Rana alticola*

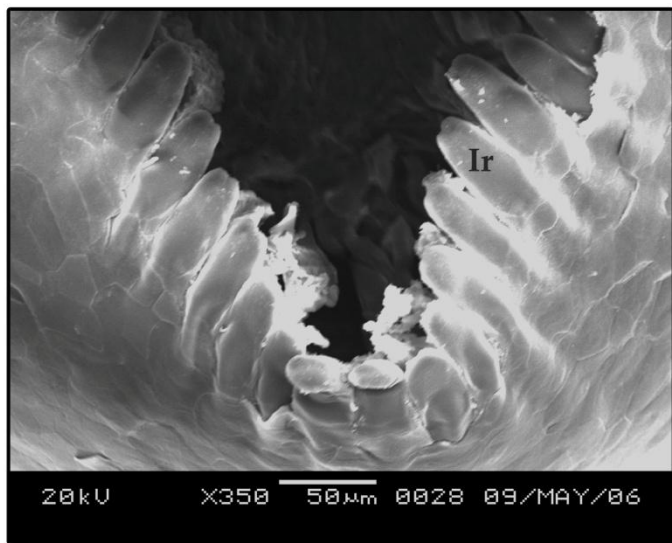


Fig 4.8: Scanning electron micrograph of the infrarostrodont (Ir) of stage 26 of *Rana alticola*

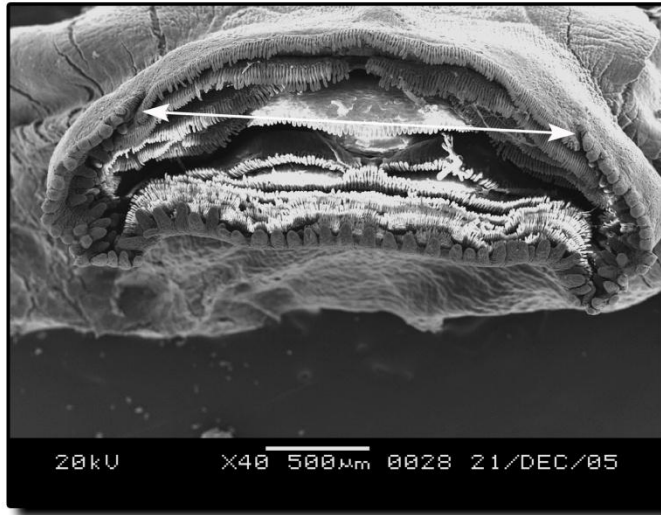


Fig 4.9: Scanning electron micrograph showing the medial papillae gap of stage 26 of *Rana alticola*

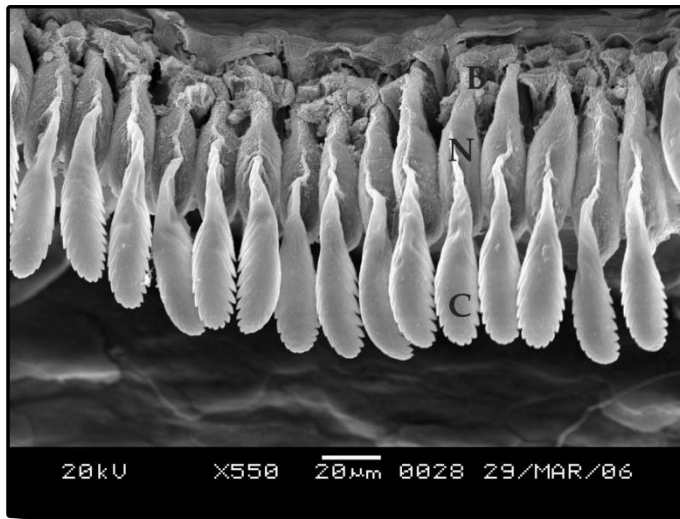


Fig 4.10: Scanning electron micrograph of labial teeth of stage 26 of *Rana alticola*. B = Base; N = Neck; C = Cusp

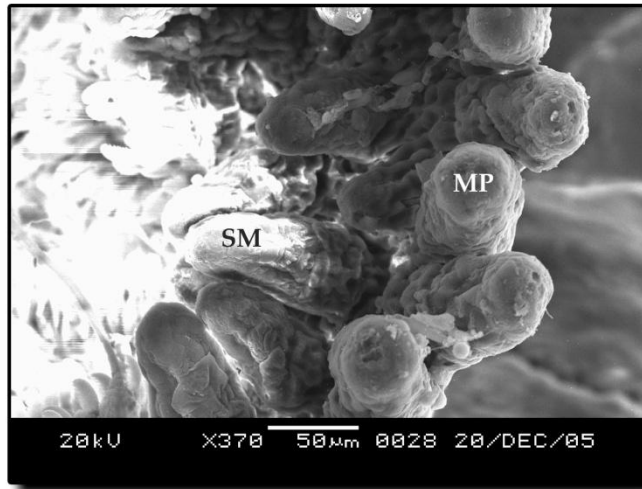


Fig 4.11: Scanning electron micrograph of sub marginal papillae (SM) and marginal papillae (MP) at stage 26 of *Rana alticola*

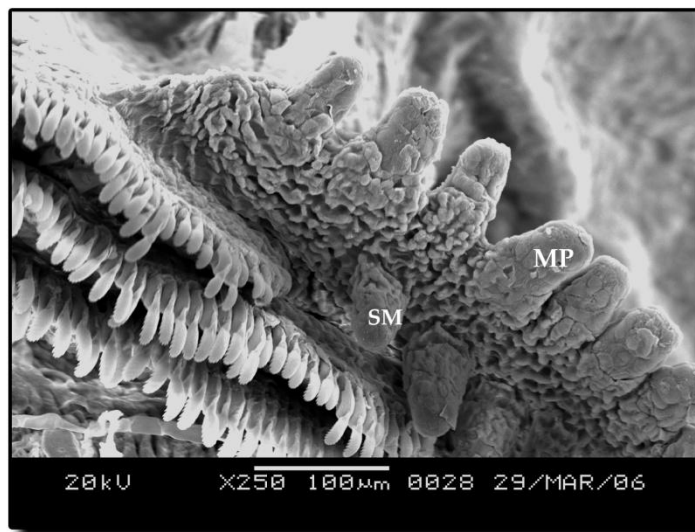


Fig 4.12: Scanning electron micrograph of sub marginal papillae (SM) and marginal papillae (MP) showing part of the labial teeth row at stage 26 of *Rana alticola*

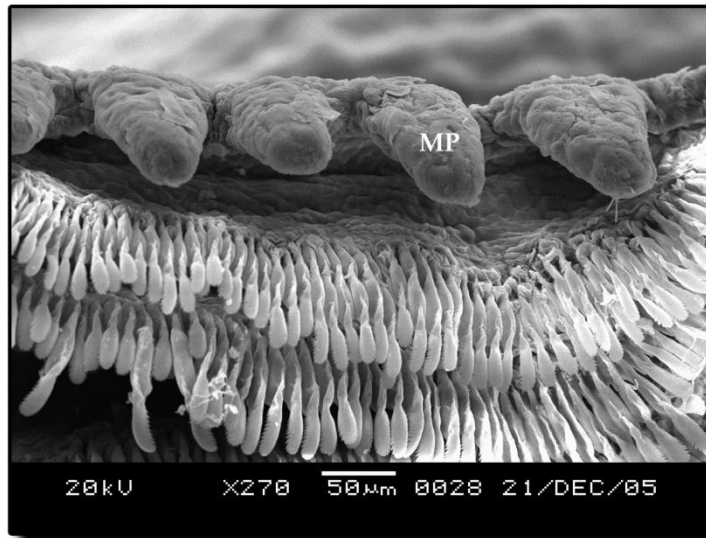


Fig 4.13: Scanning electron micrograph of marginal papillae (MP) on the anterior labium at stage 26 of *Rana alticola*

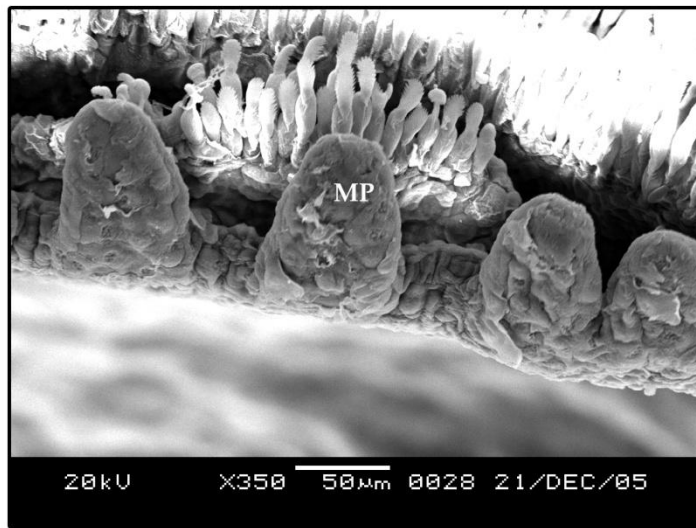


Fig 4.14: Scanning electron micrograph of marginal papillae (MP) on the posterior labium at stage 26 of *Rana alticola*

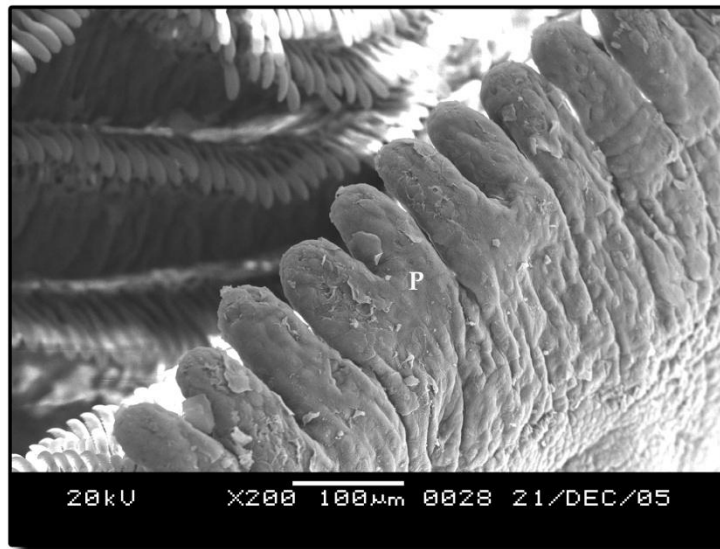


Fig 4.15: Scanning electron micrograph of bifid papillae(P) of stage 26 of *Rana alticola*

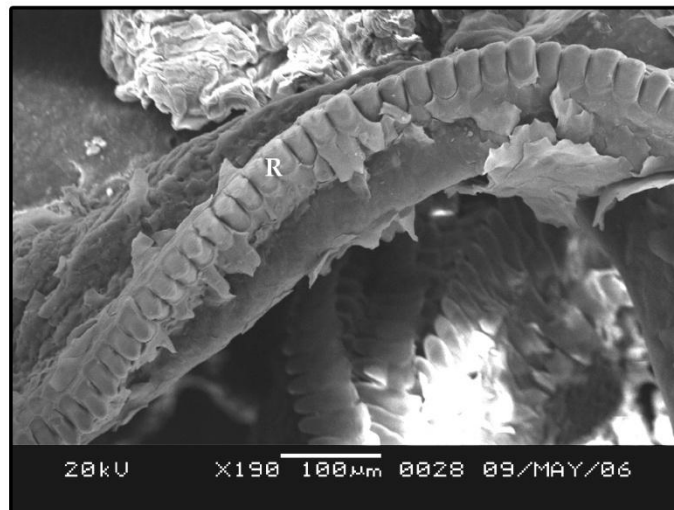


Fig 4.16: Scanning electron micrograph of mouth of stage 41 of *Rana alticola*. R=Rostrodont.

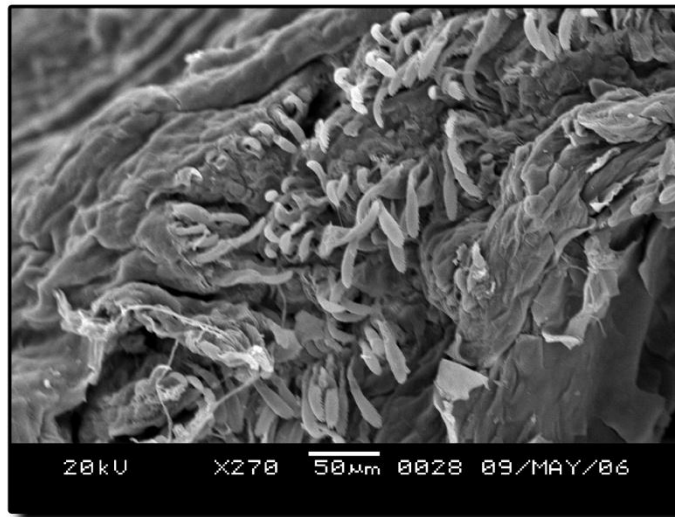


Fig 4.17: Scanning electron micrograph of degenerating labial tooth rows of stage 42 of *Rana alticola*

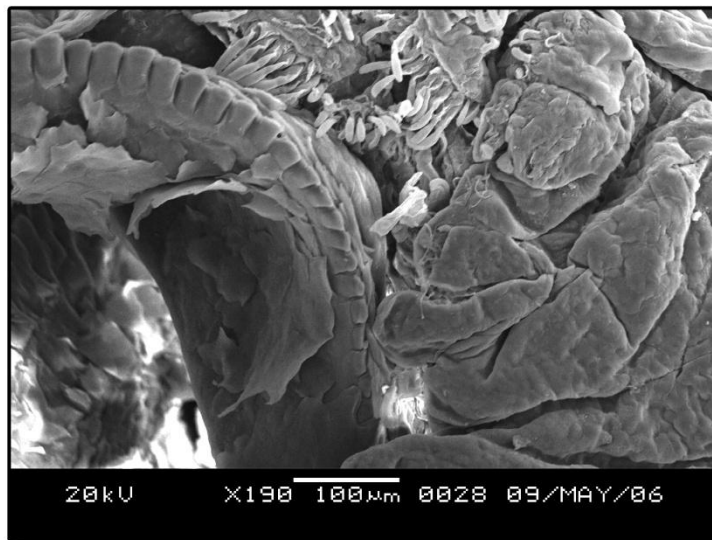


Fig 4.18: Scanning electron micrograph of degenerating papillae of stage 42 of *Rana alticola*



Fig 4.19: Scanning electron micrograph of the oral structure of stage 43 of *Rana alticola*

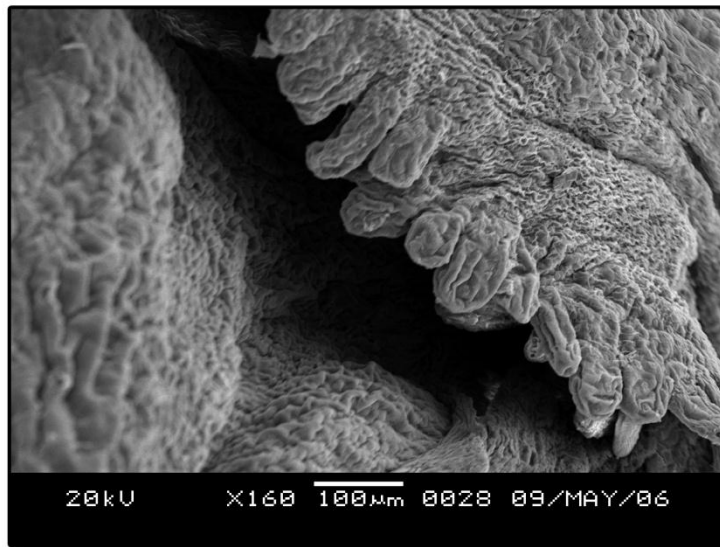
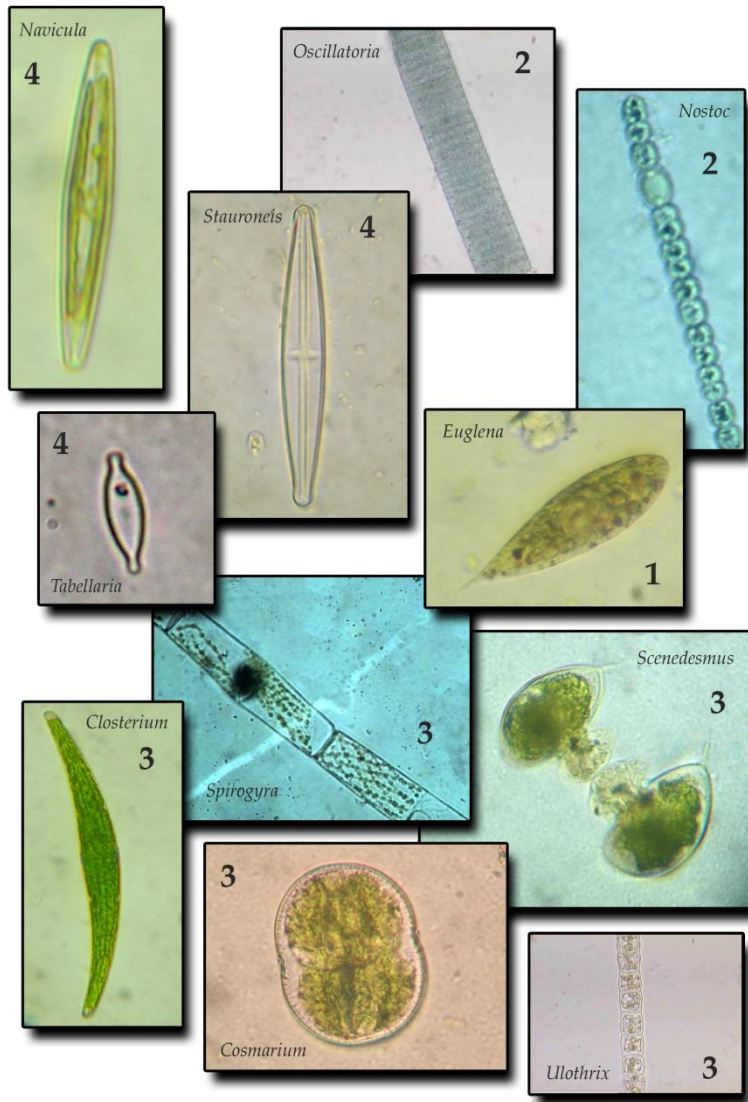


Fig 4.20: Scanning electron micrograph of the papillae of stage 43 of *Rana alticola*



Euglenophyceae: 1 Chlorophyceae: 3
 Cyanophyceae: 2 Bacillariophyceae: 4

Fig 4.21: Some Phytoplankton found in the gut of *Rana alticola* tadpole

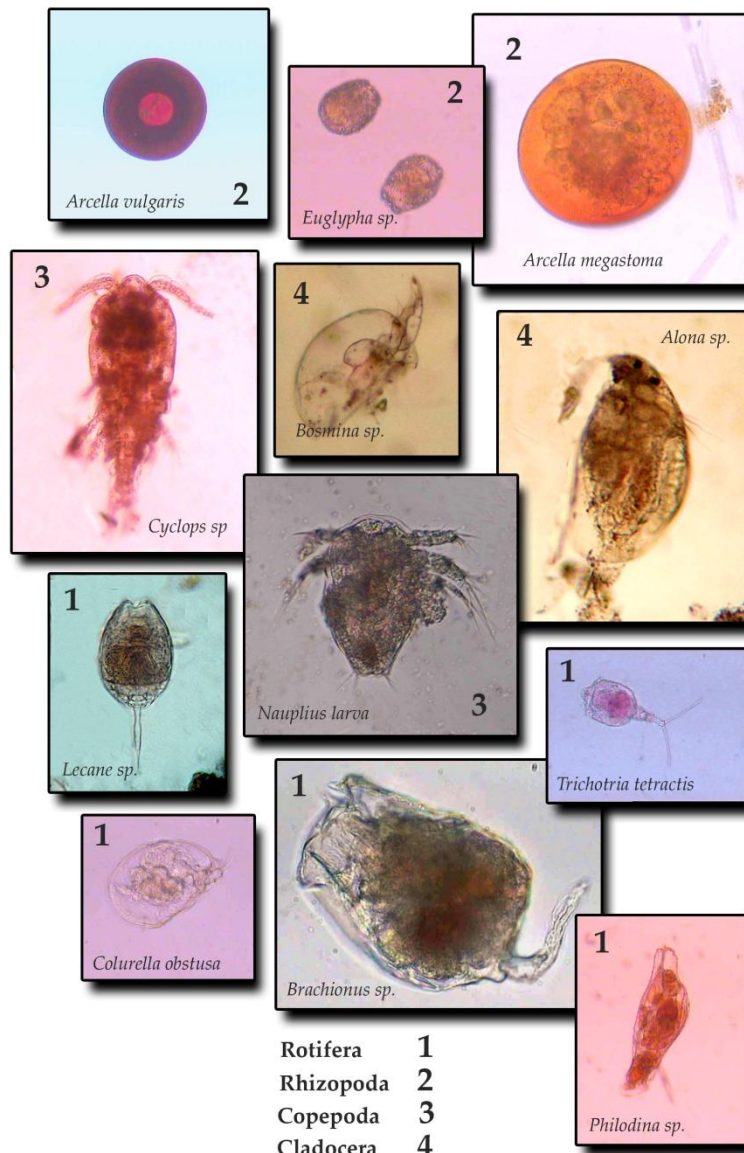


Fig 4.22: Zooplankton found in the gut of *Rana alticola* tadpole

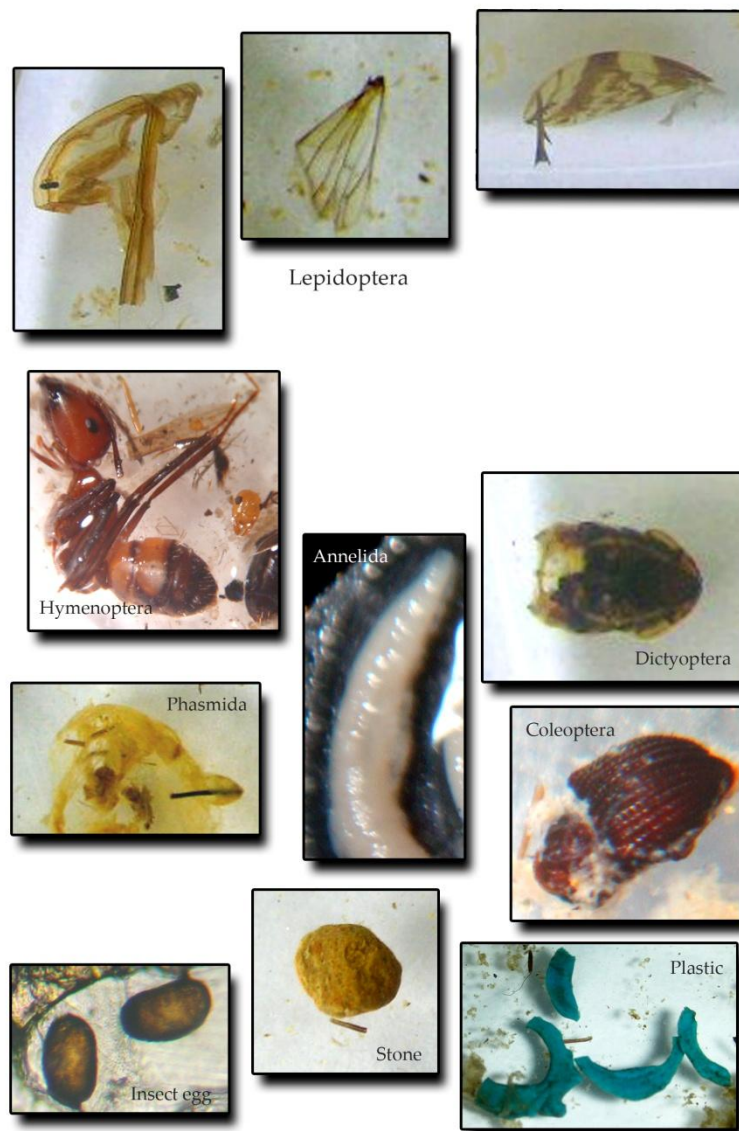


Fig 4.23: Gut contents of adult *Rana alticola*

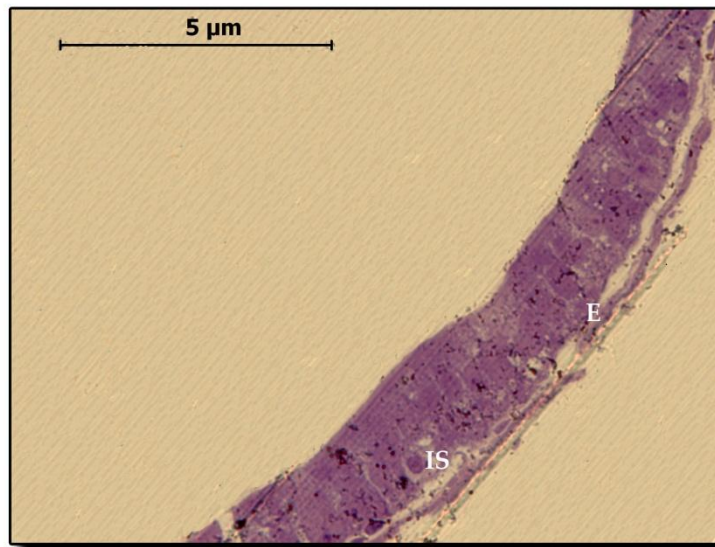


Fig 4.24: Photomicrograph of the intestine of *Rana alticola* at stage 25.
E: Epithelium; IS: Islet

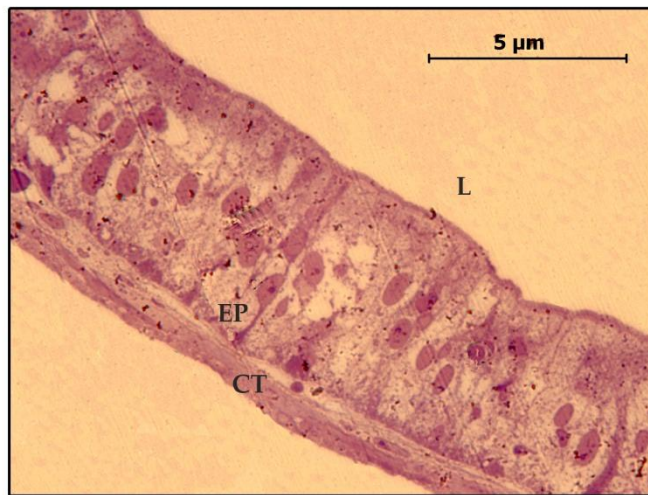


Fig 4. 25: Photomicrograph of the intestine of *Rana alticola* at stage 41:
CT: Connective tissue; EP: Epithelial cells (columnar); L: Lumen

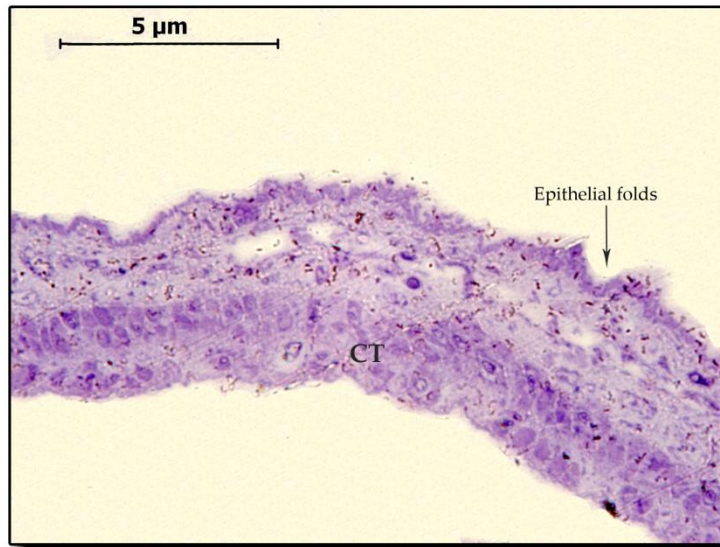


Fig 4. 26 : Photomicrograph of the intestine of *Rana alticola* at stage 42.
CT: Connective tissue

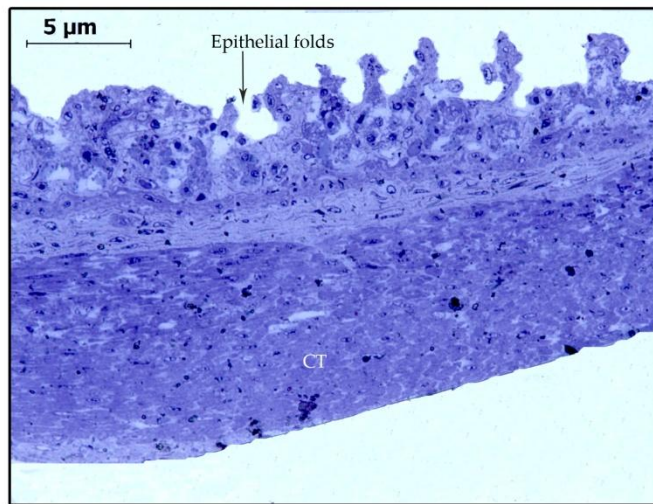


Fig 4.27: Section of the intestine of *Rana alticola* at stage 45:
CT: Connective tissue

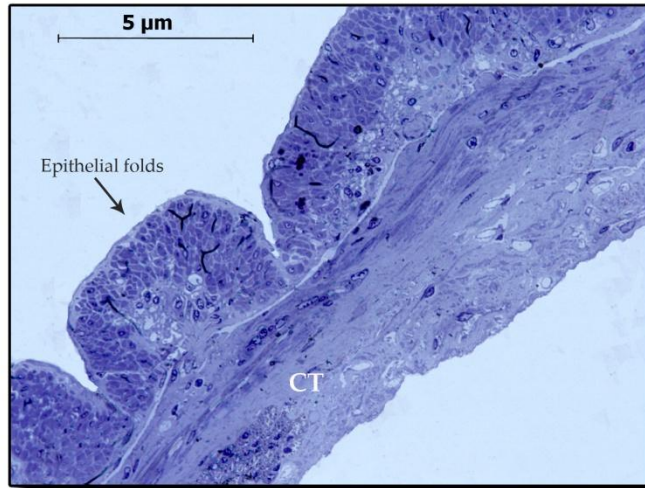


Fig 4.28: Photomicrograph of the intestine of *Rana alticola* at stage 46:
CT: Connective tissue

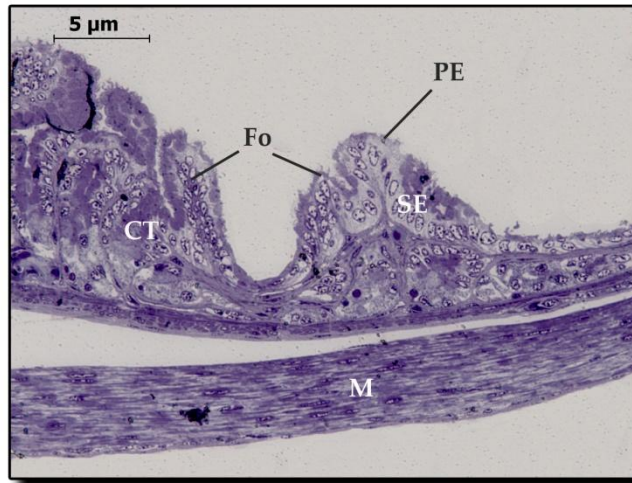


Fig 4. 29: Photomicrograph of the intestine of *Rana alticola* adult.
SE: Secondary epithelium, CT; Connective tissue, Fo: Intestinal folds,
PE: Primary epithelium, M: Muscle

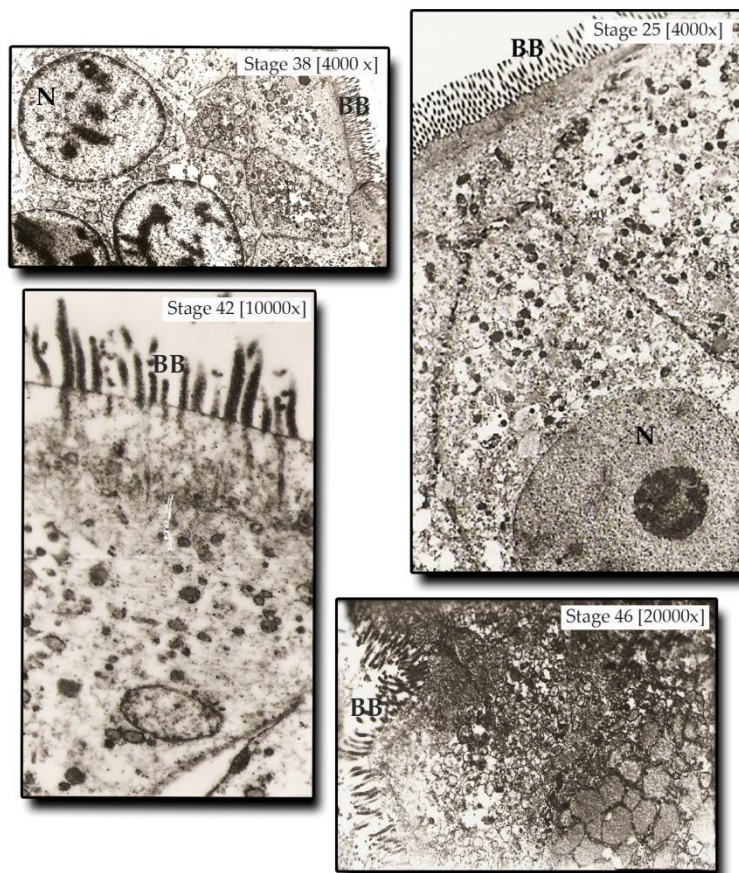


Fig 4.30: TEM micrograph of *Rana alticola* tadpole at :

Stage 25 & 38 - showing long compact brush border (BB),nucleus (N) visible
 Stage 42 & 46 - brush border less compact

CONCLUSION

During the present study and investigation with reference to the distribution of *Rana alticola* in Mizoram, it was found that the frog has a wide range of distribution and can adapt to low and high altitude ranging from 46 m asl to 1113 m asl. Different parts of Mizoram were surveyed and collections were made from all the eight districts (i.e. Aizawl, Kolasib, Champhai, Mamit, Serchhip, Lunglei, Lawngtlai and Saiha) of Mizoram. From Aizawl district, collections were made from Aizawl (743-965 m asl), Rungdil (332 m asl), Sairang (50-80 m asl), Sihhmui (180 m asl), Tamdil Lake (760 m asl), Tuirial (179 m asl) and Tuirini (298 m asl). From Kolasib District, collections were made from Buhchang (46 m asl), Herhse stream (308-326 m asl) and Kawnpui (910 m asl). From Champhai District, collection was made from Lundil (965 m asl). From Mamit District, collections were made from Lengpui (390 – 400 m asl) and Tut (74 m asl). From Serchhip District, the frogs were collected from Chhingchhip (1113 m asl), Mat (651 m) and Thenzawl (741-810 m asl). From Lunglei District, collection was made from Theiriat (1048-1060 m asl). From Lawngtlai district, collection was made from Lawngtlai (847 m asl) and from Saiha District collections were made from Khankawn (193 m asl) and New Latawh (458 m asl). Adults of *Rana alticola* were collected from permanent ponds, streams and rivers covered by surrounding vegetations and among bushes surrounding water bodies during its breeding period and it was observed that adults of *Rana alticola* were extremely rare and nocturnal but their tadpoles were abundantly available.

From the above mentioned survey sites, tadpoles were collected from all the 20 sites which are Aizawl, Rung dil, Sairang, Sihhmui, Tam dil, Tuirial, Tuirini, Buhchang, Herhse, Kawnpui, Lun dil, Lengpui, Tut, Chhingchhip, Mat, Thenzawl,

Theiriat, Lawngtlai, Khankawn and New Latawh, but adults were encountered from 12 survey sites namely Rungdil, Sairang, Tamdil, Tuirini, Herhse, Buhchang, Lundil, Tut, Chhingchhip, Theiriat, Lawngtlai, and New Latawh. It may be suggested that the tadpoles can adapt to different habitat like rivers, streams and pools. During the survey, tadpoles of *Rana alticola* were encountered during the months of August, September, October, November, December, January, February and March. The froglets were found in plenty during the months of April and May. No tadpoles were found in the months of May, June and July indicating that the tadpoles have metamorphosed. The tadpoles were usually found to inhabit and prefer a part of water where the current is slow. The tadpoles were usually found in large number.

The breeding behavior of *Rana alticola* was observed from three study sites namely Sairang River (Study site I), Herhse stream (Study site II) and Tamdil Lake (Study site III) in Mizoram. Observations in the field during the study period indicate that the breeding season of *Rana alticola* starts from late June which coincide with the rainy season and continue till early October. It was observed that the male emerged first and reaches the breeding site first, and then they start the advertisement calls which attract the receptive female towards the breeding site. During the breeding season, the males of *Rana alticola* starts calling from 1400 hours till 2100 hours from the month of June following the emergence. The call sounds like the chirping of a bird with a 'chirp' sound. The advertisement call is species specific. The call consisted of four notes and the call duration lasted for one second. Each note consists of two to three pulses and each note lasts for 0.1 second. The advertisement call is the major factor in the courtship of *Rana alticola* which

initiate the female to approach the calling male, the male will grasp the female by the armpit and amplexus will start. Amplexus is axillary where the male grasps the female at the axilla. It also gives a distress call when in captivity which differ from the advertisement call. The loud, explosive distress calls given in response to acute disturbance or grasping by a potential predator are produced by either sex or sometimes even newly metamorphosed young and are acoustically dissimilar to the advertisement calls.

An interesting behavior known as combat behavior is seen in *Rana alticola* where unpaired males attack amplexant males that females have actively chosen and, if they are larger than amplexant males, can displace them. Males always greatly outnumber females and attempt to dislodge one another from the backs of females. Hence, in the observation, a significant behavior is seen where male-male competition takes the form of caller-satellite associations, in which a male calling from a call site or breeding territory is attended closely by one or more non-calling males that seek to intercept and mate with females as they approach the calling male. Sexual dimorphism is pronounced where the female is almost double the size of male. The Snout Vent Length (SVL) of females ranges from 41.9 mm – 60.92 mm whereas the SVL of males ranges from 32.33 mm - 46.89 mm. The sex ratio in this species is always male-biased with 1:10 female: male ratio.

Oviposition sites of *Rana alticola* were found amongst leaf litter along the edge of the water bodies. The eggs were deposited in the stagnant water as well as flowing water but attached to vegetation and were always submerged in the water. The female starts depositing the eggs after some hours from the time of amplexus and amplexus can continue up to 24 hours. The eggs were deposited in multiple

clutches and the colour of the egg is light brown. The egg measures about 1.2 to 1.5 mm in diameter. The clutch size ranges from 1002 – 2018 numbers and there is no correlation between SVL of female and the clutch size.

After the breeding season, the adults are very rare in all the three study sites. The adults, if encountered during the non breeding season, were found in bamboo stumps present around the study sites. During the non breeding season, there is decrease in the level of the water at all the three study sites and a large number of tadpoles were found during this period. From April to May, only juveniles of *Rana alticola* were seen around the study sites, and no breeding activities were seen during these months. No adults were seen at all but a large number of metamorphosed froglets were observed during these periods. The metamorphosed froglets were seen in a huge number among the vegetations in the water, on bamboo stumps, between rock crevices and on the rock boulders present around the study sites.

The histological study of the testis under a compound microscope revealed that, during the breeding season, mature spermatozoa occur in a cluster with their tail extending into the lumen of the tubules. The size of the ovary during the breeding period varies depending on the maturity of the ova in the ovary and there are several lobes on each side of the ovary. Both young and mature oocytes were found to be present during the breeding period.

During the breeding season, i.e., from June to October in all the three study sites, the rainfall ranged from 67 mm to 704 mm. The relative humidity ranged from 24% to 98%. The air temperature recorded was 16°C to 35°C. The water temperature ranged from 13°C to 25°C and pH ranged from 6.5 to 7.5.

The complete developmental time of *Rana alticola* takes 215 days which is approximately seven months. During the period of development, rainfall ranged from 1.2 mm to 611.4 mm, the relative humidity ranged from 26% to 95%, air temperature ranged from 12°C to 35°C, water temperature ranged from 10°C to 25°C and the pH ranged from 6.4 to 7.6. Hatching takes place at stage 19 (i.e. heartbeat stage) after about 13 days and 20 hours, which is comparatively late as compared to other ranids. 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.

Rana alticola belong to the lotic-benthic ecomorphological guild of Altig & Johnston (1989). The oral structures of the tadpole of *Rana alticola*, when observed under the stereoscopic binocular microscope, revealed that, the oral disk is large, anteroventral, not laterally emarginated, directed ventrally. The keratodont starts to appear from stage 25. The mouthpart at stage 25 is simple with labial tooth row formula (LTRF) which is not consistent; and may vary from 1- 2: (2+2) - (4+4) / 1+1: 3-5. The present study established that *Rana alticola* tadpoles have marginal teeth which make their first appearance at stage 26. The number of tooth rows or keratodont rows increases during ontogeny. However, the LTRF at these stages are not consistent and it may vary from 2: (3+3) - (5+5)/ 1+1: 4-7. The LTRF remains the same from stage 26 through stage 41. The oral structure starts to degenerate

when the forelimb emerged at stage 42. By the time the tadpole reach stage 43, the labial tooth row has disappeared completely.

Scanning electron microscopic studies of *Rana alticola* tadpoles revealed that, the oral disc is composed of anterior (upper) labium and posterior (lower) labium. Marginal papillae is present on the edge of the oral disc with a wide dorsal gap, sub marginal papillae is also present. The upper jaw sheath is straight with the suprarostrodont which appeared to be serrated and pointed while the lower jaw sheath is a central V-shaped groove with the infrarostrodont which are serrated and blunt and the mouth is present in between. The tooth rows are uniserial and the labial tooth is keratinized and each keratinized labial tooth is derived from cells in the base of the tooth ridge and consists of three indistinct regions; a distal head with around 20 terminal cusps, an intermediate body known as the neck and a basal hollow sheath known as the base. Submarginal papillae are positioned in lateral parts of upper and lower labia, both the marginal and submarginal papillae has a rounded tip and the papillae are closely spaced, short and numerous in number At some point, bifid papillae are also seen in the tadpole of *Rana alticola*.

Qualitative analysis of gut contents of the tadpoles and adults of *Rana alticola* was done and observations revealed that tadpoles started feeding from stage 25 onwards. The gut contents of the tadpoles at stage 25 mainly include phytoplankton like Euglenophyceae, Cyanophyceae, Chlorophyceae and Bacillariophyceae and as the development progresses, the gut contents were mainly zooplankton like Rotifera, Rhizopoda, Cladocera and Copepoda. Cannibalism was observed in the tadpole of *Rana alticola* during the time of food shortage in the laboratory condition. Observation of the gut contents of the adult frog of *Rana*

alticola revealed that the diet of the adult frog mainly includes insects. Apart from insects, the diet also includes annelids, crustaceans, fingerlings and some plant materials. Insects include the order Isoptera (e.g. Termites), Lepidoptera (e.g. butterfly), Hymenoptera (e.g. ants), Coleoptera (e.g. beetle), Orthoptera (e.g. grasshopper), Hemiptera and Dipteran flies. On some instances plastic materials, sand particles and pieces of small stones were also found in the gut of the adults.

As the tadpole starts feeding from stage 25, study of the ultrastructural sections of the intestine was done for stage 25, 38, 42 and 46 and the study revealed that the brush border appears to be long and compact at stage 25 and 38 while it is less compact at stage 42 and 46. Histological studies of the intestine at stage 25, 41, 42, 45 and 46 revealed that the intestine is long and simple from stage 25 to stage 41. It consists of a single layer of columnar epithelium surrounded by thin layers of muscles with little intervening connective tissue. From stage 42 onwards, the intestine begins to shorten and the epithelial folds start to form. The intestine at stage 46 resembles that of the adult. A histological study of the intestine of the adult reveals that it has elaborate connective tissue and muscles. The primary epithelium degenerates and the secondary epithelium are formed. The epithelium also forms multiple circular folds; and these intestinal folds appear as several circular folds that run longitudinally and are straight along the gut axis, gradually increasing in number and height, and finally being modified into longitudinally zigzagged folds. The zigzag folds then remain throughout adulthood.

Hence, the present investigation conducted in Mizoram, North East India, contribute more and new informations on the distribution, habit and habitat, breeding behaviour, development and food and feeding behaviour of *Rana alticola*.

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CURRICULUM VITAE

1. Name : *Saipari Sailo*
2. Father's name : *Sailothanga Sailo*
3. Date of birth : 31st March 1978
4. SC/ST/OBC : ST
5. Permanent Address: Y-1/5, Chhinga Veng,
Aizawl-796001,
Mizoram

6. Academic Record

| Examination | Division | Year | Board/Univ. |
|-------------|----------|------|---|
| H.S.L.C | I | 1993 | M.B.S.E |
| P. U (Sc.) | II | 1996 | Dept. of Pre- Univ. Education, Bangalore. |
| B. Sc | I | 2000 | N.E.H.U |
| M. Sc | I | 2002 | N.E.H.U |

ACHIEVEMENTS:

- Best Participant at the SERC 1st School in Herpetology held at North Orissa University from 27th December 2007 to 8th January, 2008.
- Best Oral Presentation at The National Symposium on Advances in Zoology: Faunal Diversity and Ecophysiology organized by Department of Zoology, North Eastern Hill University, from 13th to 14th March, 2008.

ORAL PRESENTATIONS:

- **Presented a paper** entitled “ Amphibian fauna of Tamdil Wetland, Mizoram, North East India” at the UPE-Bioscience Seminar on Assessment, Conservation and value addition of Biodiversity, organized by School of Life Sciences, NEHU, Shillong-22 from 22nd to 23rd April, 2010.
- **Presented a paper** entitled “Anuran Diversity in Mizoram” at the UGC sponsored National Seminar on Biodiversity Spectrum of North-East India organized by Department of Zoology, Arya Vidyapeeth College, Guwahati in collaboration with Zoological society of Assam from 18th to 19th September, 2009.
- **Presented a paper** entitled “Habitat destruction and its impact on Amphibians” at the Regional seminar on Environmental Problems: Extinction of Biological Species in North-East India organized by Department of Zoology; Synod College, Shillong on 17th September, 2009.
- **Presented a paper** entitled “A note on the breeding behavior of some anurans in Mizoram” at the 19th All India Congress of Zoology; National Zoology Congress seminar on “Biodiversity and Human Welfare” held at the Department of Zoology, Gauhati University, from 29th to 31st December, 2008.
- **Presented a paper** entitled “Amphibian Diversity in Mizoram” and awarded the second Best Oral Presentation at the National Symposium on Advances in Zoology: Faunal Diversity and Ecophysiology organized by Department of Zoology, North Eastern Hill University, from 13th to 14th March, 2008.
- **Presented a paper** entitled “Anuran fauna of Mizoram” at the “Regional Symposium on ‘Current Research Thrust in Animal Sciences: Interface with End Use Researchers and Stake Holders” at North Eastern Hill University, Shillong, from 15th to 16th March, 2007.
- **Presented a paper** entitled “Studies on the breeding and development of *Rana nicobariensis* (Stoliczka, 1870) (Anura : Ranida)” at the “Regional Symposium on Research Thrust in Animal Sciences in N.E. region – an appraisal” at North Eastern Hill University, Shillong, from 24th to 25th March, 2006.

POSTER PRESENTATION:

- **Poster presentation** entitled “Studies on the breeding behavior and development of *Nasirana alticola*” at the 96th Indian Science Congress held at North Eastern Hill University, Shillong, from 3rd to 7th January, 2009

SEMINARS / SYMPOSIUMS / WORKSHOPS ATTENDED:

- Attended National Seminar on Human Security in North-East India: Problems, responses and strategies organized by Mizo Research Scholars' Association and Government Zawlnuam College, Mizoram at Information & Public Relation Hall, Aizawl, Mizoram from 30th to 31st March, 2009.
- Attended workshop on High Performance Computing organized by C-DAC, Pune and NEHU; Computer Centre, Shillong from 3rd to 6th March, 2009.
- Attended workshop on Statistical method in Medical and Health Sciences organized by Statistics and Mathematics Unit Indian Statistical Institute, Kolkata and Statistics Department, North Eastern Hill University, Shillong from 19th to 21st February, 2009.
- Attended the SERC 2nd School in Herpetology held at the Wildlife Institute of India, Dehradun, India, from 1st to 14th September, 2008.
- Attended the SERC 1st School in Herpetology workshop at North Orissa University and awarded the Best Participant Award, from 27th Dec 2007 to 8th January, 2008.
- Participated in the XVIII National Symposium on Chronobiology at North Eastern Hill University, Shillong from 8th to 10th November, 2006.
- Participated in Conservation beyond Boundaries: A training Programme for Conservation Biologists at Manas National Park, Assam organized by Aaranyak in collaboration with British Council, from 23rd to 27th February, 2006.
- Attended "Training Programme on AAS and HPLC" at the Sophisticated Analytical Instrument Facility, North Eastern Hill University, Shillong, from 7th to 9th December, 2005.
- Attended National Symposium on Developmental Dynamics organized by Department of Zoology, University of Kalyani in collaboration with Indian Society of Developmental Biologist, Pune, from 23rd to 25th November, 2005.
- Attended "Short Term Course on Electron Microscopy" conducted by the electron microscope division of the Sophisticated Analytical Instrument Facility, NEHU, from 22nd to 30th September, 2003.

PUBLISHED PAPERS

- Sengupta, S., **Sailo, S.**, Lalremsanga, H. T., Das, A., Das, I. 2010. A new species of *Leptolalax* (Anura: Megophryidae) from Mizoram, north-eastern India. *Zootaxa* 2406: 57-68.
- Sailo, S.**, Lalremsanga, H. T., Hooroo, R. N. K., Lalrotluanga, and Ohler, A. 2009. *Ingerana borealis* (Annandale, 1912): a new record from Mizoram (India), with notes on its systematic position and natural history. *Alytes* 27(1): 1 – 12.
- Sailo, S.**, Lalremsanga, H. T., and Hooroo, R. N. K. 2007. *Amolops kaulbacki* (Kaulback's Torrent Frog): New record for India. *Herpetological Review* 38(1): 96.
- Lalremsanga, H. T., **Sailo, S.**, and Hooroo, R. N. K. 2007. *Leptobrachium smithi* (Smith's Litter Frog): New state record for Mizoram, India. *Herpetological Review* 38(1): 98.
- Lalremsanga, H. T., **Sailo, S.**, and Hooroo, R. N. K. 2007. Record of *Rana chloronota* (Gunther, 1875) (Anura: Ranidae) from Mizoram, northeastern India. *Hamadryad* 31(2): 361 – 362.
- Lalremsanga, H. T., **Sailo, S.**, Kharbuli, B., and Hooroo, R. N. K. 2007. Record of *Sylvirana leptoglossa* (Cope, 1868) (Anura: Ranidae) from Kolasib district, Mizoram, northeastern India. *Froglg* 13: 9 – 10.
- Sailo, S.**, Kharbuli, B., and Hooroo, R. N. K. 2005. Record of *Kaloula pulchra* (Gray, 1831) from Mizoram, northeast India with notes on its burrowing behaviour. *Cobra* 62: 25 – 28.