

**STUDIES ON THE ECOLOGY, BREEDING BEHAVIOR
AND DEVELOPMENT OF RANID AND MICROHYLID
ANURANS PREVALENT IN MIZORAM, NORTHEAST
INDIA.**

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IN MIZORAM, NORTHEAST INDIA.**

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DECLARATION

I, H. T. Lalremsanga, 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

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Candidate

***Dedicated to my beloved
parents***

CHAPTER 1

INTRODUCTION

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INTRODUCTION

Among amphibians, the order Anura constitute the vast majority (88%) of living species of amphibians and the bulk of their genetic, physiological, ecological, and morphological diversity, which is represented by a total of 5227 species under 32 families and ca.372 genera are known from the world (Frost *et. al.*, 2006). The word amphibian is derived from the Greek words “amphi” meaning “both” and “bios” meaning “life”. Nearly all amphibians live the first part of their lives in water and the second part on land. There are 5948 recognized species of amphibians. The living amphibians that exist today are divided in to three orders: Caudata or Urodela (salamanders), Gymnophiona or Apoda (Caecilians-legless, burrowing amphibians that live only in the tropics and are not often seen because of their secretive habits) and Anura or Salientia (frogs, toads and their relatives).

Anurans are present on all continents with possible exception of Antarctica (Just *et. al.*, 1981; Duellman and Trueb, 1994). Most species can be found in the temperate regions. More than 80 percent of them live in the tropics and subtropics, but they can also thrive in higher mountainous regions and in dry, desert climates (Lamm *et. al.*, 2003). To live in such a variety of habitats, they have special adaptations to help them survive. According to Inger (1966), the major factors that ultimately limit the distribution of tropical amphibians are ecological factors, such as temperature, total rainfall, vegetation and competition, geographical and geological factors. Topography may influence amphibian composition and diversity indirectly (Pearman, 1997). Amphibian diversity and abundance varies significantly among

neotropics, Africa and Southeast Asia which has been attributed to differences in litter fall rates, mast fruiting, heterogeneity within regions, breeding habitat constraints, and geological history (Allmon, 1991). They are a perfect indicator species for many reasons. They live both in water and on land. Their permeable skin absorbs water, oxygen, and other chemicals from the environment. They also consume both plant and animal matters. Therefore, they are more susceptible to minute changes in the environment (pollution, weather changes, etc.). Initially, these changes may only affect frog and other amphibian populations, but over time, they may have a negative effect on humans and other animal species. Amphibians are integral components of many ecosystems, often constituting the highest fraction of vertebrate biomass (Burton and Likens, 1975; Beebee, 1996). Amphibians play keystone roles in forest ecosystems, influencing vital ecosystem processes and contributing to system resilience-resistance (=stability) (Davic and Welsh, 2004). Adult amphibians are important predators as well as prey and larval amphibians may be important herbivores (Blaustein *et. al.*, 1994). Therefore, a world-wide decline of amphibian populations could have a significant and detrimental impact on both natural ecosystems and human welfare (Gardner, 2001).

Amphibians exhibit a wide range of reproductive cycles in response to changes in environmental conditions (Houck and Woodley, 1995). Three major environmental factors have been implicated in the regulation of the amphibian breeding cycle: rainfall, photoperiod, and temperature (Lofts, 1974). Temperature and photoperiod play important roles in the timing of the reproductive cycle (Zug, 1993; Huang *et. al.*, 1997). Many anuran species in aseasonal tropical environments are able to reproduce throughout the year (Duellman and Trueb, 1994), whereas in

seasonal environments, breeding activity of most anuran is associated with the rainy season both in the temperate zones and the tropics (Huang *et. al.*, 1996, 1997). Rainfall acts as the most proximate stimulus for the breeding behavior of amphibians in both temperate and tropical zones (Pough *et. al.*, 1998). Although temperate anurans respond to rainfall, temperature seems to be a major factor initiating breeding activity, as evidenced by geographic variation in the time of breeding. The social behaviors of most anurans are associated with acoustic communication in the form of vocalization (Krishna and Krishna, 2005). Sound production is primarily a reproductive function of male anurans. Advertisement calls attract females to breeding areas, and announce to other males that a given territory is occupied. Advertisement calls are species specific. The intra-specific diversity in call characteristics allows females to discriminate among potential conspecific mates based on some of the same acoustic parameters used for species identification (McClelland *et. al.*, 1996). Although most anurans are normally solitary, they often come together in large breeding aggregations during the spring or rainy season.

Once the males and females have found each other, mating can occur. The males who are usually smaller than females, climb on to the females' back and clasp the female from above, which is known as amplexus. Fertilization is always external and occurs during amplexus, which can take hours or days depending on the species. Eggs are laid individually, in clumps or in strings, and the number of eggs deposited can range from many thousands to less than twenty (Lamm *et. al.*, 2003). Most eggs hatch into aquatic larvae i.e. tadpoles. Oviposition and fertilization are accomplished by synchronized movements by both partners. In anurans in which the pair is in amplexus prior to oviposition, the female usually chooses the oviposition site. Some

amphibians lay their eggs and let them develop by themselves while others lay eggs and guard them. In this life phase the tadpole develops quickly by feeding mainly on algae, plankton and dead animal matter found on the bottom of ponds, lakes and streams (Urban, 2005).

Many amphibians metamorphose, or change from gill breathing aquatic larvae to air-breathing semi-aquatic terrestrial adults. Metamorphosis is a post embryonic period of profound morphological changes by which the animal alters its mode of living. The morphological changes associated with metamorphosis in amphibians represent the change from an aquatic to a terrestrial mode of life. This variability of amphibians makes them one of the most interesting group to study, and amphibians have indeed contributed a great deal to science. They are most commonly used for studies anatomy, reproduction and hormones. Frog eggs have been very popular material for embryological studies because of external fertilization, large clutch size and relatively large size of the eggs. They have no shells or other opaque covering; therefore one can readily watch them developing in a little pond water. They do not need to be maintained at special temperature.

In addition, the metamorphosis of the mainly herbivorous aquatic tadpole into the carnivorous amphibious froglet is one of the most fascinating transformations in development. In contrast to the other two recent amphibian orders (salamanders and caecilians), frog larvae are usually filter-feeding omnivores (Grosjean *et. al.*, 2004). They have a mechanism from extracting suspended particles of food from water (Kenny, 1969; Severtzov, 1969; Wassersug, 1976). Tadpoles possess morphological specializations that are related to the size or kind of food ingested. Their specialized feeding habits require mouthparts and a digestive system

that is typically different from the adult frog. Due to opportunistic dietary habits, tadpoles occupy an important position in lower trophic level, and are main constituents of food chain in pond ecosystem (Khan and Mufti, 1994). Anurans are traditionally described as generalist predators with opportunistic foraging behavior (Santos *et. al.*, 2004). Most adult anurans have adopted a sit-and-wait strategy. However, the strategy used by an individual may vary with the abundance of prey.

Out of 32 families under the order anura, the family Ranidae is represented by ca. 54 genera with 772 species (Frost *et. al.*, 2006). Most Ranidae are streamlined, with bullet-shaped bodies and pointed heads, large eardrums, protruding eyes; on most species of this family, the fingers are perfectly free, but toes are more or less completely webbed (Chanda, 2002), and well-developed legs that enable them to make prodigious leaps when escaping from danger. 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). The family exhibits tremendous ecological, morphological, and developmental diversity across its wide geographic range. The true frogs of family ranidae, have the widest distribution of any frog family. They are found all over North America; in Central America and in the northern part of South America; in all of Europe, Asia and Africa excepting frozen or desert areas; and in northern Australia, but not in southern Australia or New Zealand (Cochran, 1967). The true frogs vary greatly in size, ranging from small, such as the Wood frog (*Rana sylvatica*), to the largest frog in the world, the Goliath frog (*Conraua goliath*). Many of the true frogs are aquatic or habitat close to water. Most species lay their eggs in

the water and go through a tadpole stage. However, as with most families of frogs (Ranidae), there is large variation of habitat within the family (Noble, 1931).

The family Microhylidae (narrow-mouthed toads) is represented by 69 genera and 432 species, which is the largest number of genera out of any frog family. It includes some of the most highly specialized of all salientia. Microhylids are characterized by absence of keratodonts and keratinized jaw sheaths in the larval stage; position of spiracle in the median posterior; the cartilage which supports the upper jaw of most tadpoles is absent; the lower lip is a series of folds, which in some species may protrude well beyond the mouth; the external nostrils do not appear until late in the larval development. Vomerine teeth are absent and jaws without tooth in adult. Terminal phalanges of fingers and toes are simple or T-shaped (Cochran, 1967; Chanda, 2002 and Haas, 2003). In addition, adults have two or three palatal folds (Heying, 2003). Most species are brown, tan, or yellow-brown on their backs, sometimes with brighter colors on their underside. As suggested by their name, microhylids are mostly small frogs. Many species are below 15 millimeters in length, however some larger reach 80-90 millimeters (Cogger *et. al.*, 2004). The members of this family live in many different habitats. Microhylids are found in arid deserts, extremely wet forests, and almost everywhere in between. Most of the subfamilies represent a radiation within a limited geographical area. Some are predominantly tree frogs, terrestrial and semi-fossorial (Heying, 2003). They display a variety of reproductive strategies including aquatic larvae, larvae without mouthparts and direct development from egg to frog (AmphibiaWeb, 2008). Distribution is widespread, and microhylids are found throughout most tropical and temperate regions except for the Palaearctic, most of Australia, the West Indies, and

most Oceanic islands. Major radiations, and most of microhylid diversity, are found in Madagascar and New Guinea (Duellman and Trueb, 1994; Heying, 2003).

The microhylid genus *Microhyla* is distributed from India and Sri Lanka to China, Taiwan, and the Japanese Ryukyu Archipelago, and southeastwards to the Greater Sunda Islands, Bali, and the Philippine Sulu Archipelago (Dehling, 2010). *Microhyla* is the only genus found in both hemispheres (Cochran, 1967). The genus *Microhyla* is composed of 30 species (AmphibiaWeb, 2009). Dubois (1987) divided *Microhyla* into two sub-genera, *Microhyla* and *Diplopelma*, distinguishable primarily by the terminal digital disks with median longitudinal grooves and T-shaped distal phalanges (present in *Microhyla*, absent in *Diplopelma*). By this definition, the subgenus *Diplopelma* included the species: *Microhyla okinavensis*, *Microhyla ornata*, *Microhyla picta*, *Microhyla pulchra*, and *Microhyla rubra*. Dubois (1987) also divided the subgenus *Microhyla* into two species groups, the *berdmorei* group and the *achatina* group. In the *berdmorei* group (*Microhyla annamenis*, *Microhyla annectens*, *Microhyla berdmorei*, *Microhyla fowleri*, *Microhyla mixtura*, *Microhyla palmipes*, *Microhyla perparva*, *Microhyla petrigena*, and *Microhyla superciliaris*) the palatines are present, digital disks well developed, and webbing considerable, whereas in the *achatina* group (*Microhyla achatina*, *Microhyla chakrapanii*, *Microhyla fusca*, *Microhyla heymonsi*, and *Microhyla zeylanica*) the palatines are absent, and the cartilage of the posterior portion of the nasal capsule is partially ossified, the digital disks are small, the webbing is reduced, and the tadpoles possess funnel-shaped mouths.

The genus *Kaloula* is at present known to contain 16 nominal species (AmphibiaWeb, 2009), its members distributed from Sri Lanka and India, east

through southern China and Indo-China, Indo-Malaya and the Philipines (Frost, 1985).

The abundance of amphibian species is very much uneven in India, the highest concentration of genera and species being represented in the northeast region and Western Ghats. It therefore appears that a careful analysis of the patterns of distribution of these sensitive amphibians might give us better insights into understanding how closely organism are tied to their habitats and other environmental habitats. The Indian sub-continent is an admixture of Gondwana relicts, Oriental, Ethiopian and Palearctic elements, it is clearly reflected in the diversity of amphibian fauna in the region (Vasudevan, 2007). At present, 311 species of amphibians (composed of three orders- Anura, Gymnophiona and Caudata) are known from India. Of these, 277 species belongs to Anura, 33 species belongs to Gymnophiona and 1 species belongs to Caudata (Dinesh *et. al.*, 2010) that have a very high representation of endemics, with two major centres of distribution, north-east India and the Western Ghats. Nearly sixty-three (63%) of the amphibians are endemic to India. Western Ghats is the richest region in India in terms of amphibian endemicity. Ninety three taxa are endemic to this biogeographic region with two more taxa sharing their distribution with adjacent areas. Northeastern India, which has a very high diversity among amphibians does not have many endemics within the Indian context because of the jagged political boundary of the country.

The Northeast India is the centre of attention as an area of global importance due to its rich biodiversity and is included under two biodiversity hotspot areas, namely Eastern Himalaya and Indo-Myanmar. The amphibian fauna of the Northeast India is highly diversified and at present, more than 105 amphibian species are found

in this region (Ahmed *et. al.*, 2009). Moreover, it supports some of the biologically richest areas in the World, which affords its recognition as an area of global importance. Despite its importance, this region has remained poorly explored, and all evidence suggests much of the region's biodiversity is being lost without even being recorded (Pawar, 1999).

Mizoram is part of northeast India, an important part of the Indo-Myanmar biodiversity hotspot, houses a large number of flora and fauna. Mizoram is situated in North-eastern part of India between 21°56' N - 24°31' N latitudes and 92°16' E - 93°26' E longitudes (Fig. 1). Being sandwiched between Bangladesh and Myanmar its location is of strategic significance geographically and politically; and shares a total common international boundary of about 585 kilometres with these two countries. Mizoram has a total geographical area of 21,081 square kilometers. It is bounded on the north by Cachar district of Assam and the state of Manipur; on the east and south by Chin Hills of Myanmar; on the west by Chittagong Hill tracts of Bangladesh and the state of Tripura. The state is divided into eight administrative divisions/districts – Aizawl district, Champhai district, Kolasib district, Lawnglai district, Lunglei district, Mamit district, Saiha district and Serchhip district (Fig. 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. Pachuau (1994) and Singh (1996) have given a succinct description of the physiography of Mizoram. 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 m – 1550 m), occupying most of the state and the mountainous terrain province (altitudinal range of 400 m – 2157 m) restricted to an eastern longitudinal strip adjoining Myanmar. The slopes range from straight (10° - 15°) to, very steep (>40°) (Singh, 1996). Flat lands cover only 2.4% of the geographical area. In addition, many of the ranges bounding river valleys along the eastern side are low in elevation (ca. 100 m - 500 m asl), having gentle slopes, with small stretches of flat land along either side of the river (Pawar, 1999). The average temperature varies between 11°C and 23°C in winter and climbs up to 21°C and 31°C in summer months. The monsoon season stretches from mid May to mid October. The annual rainfall is about 250 cm. The relative humidity is quite high during monsoon season. It has an abundant growth of vegetation. Out of the total geographical area (21,081 sq.km), as much as 15, 955 sq.km is covered by vegetation which accounts for about 75 percent of the area of the state. Its tropical location, which furnishes conductive climatic condition such as an adequate rainfall, moderate temperature etc. favours the luxuriant growth of vegetation. The type of vegetation which thrives in Mizoram ranges from tropical trees to sub-tropical trees (Pachuau, 1994). Some species of anurans belonging to the family Bufonidae, Dicroglossidae, Megophryidae, Microhylidae, Ranidae and Rhacophoridae have been reported from Mizoram.

It is evident from the literature that earlier work in the state of Mizoram has been mainly concentrated on the survey of the amphibian fauna (Pawar and Birand, 2001; Deuti and Dutta, 2002; Sen, 2004; Dey and Ramanujam, 2003; Ramanujam, 2005; Lalremsanga *et. al.*, 2007a, 2007b and 2007c; Sailo *et. al.*, 2005, 2007 and 2009, Sengupta *et. al.*, 2010). Although some species of Ranidae and Microhylidae

have been reported from Mizoram, there is practically no information on the distribution, ecology of habitat, breeding, development and metamorphosis of these amphibians in Mizoram. Therefore, the present investigation was conducted to know about the ecology, breeding behavior and development of some species of Ranidae and Microhylidae which are prevalent in Mizoram.

The major objectives of the proposed study were as follows:

1. Distribution of ranid and microhylid anurans prevalent in Mizoram.
2. Study of the breeding behavior like spawning, habit and habitat, and the ecological factors (temperature, pH, rainfall and relative humidity).
3. Study of developmental stages with reference to morphometric changes of the tadpoles.
4. Study of food and feeding behavior of tadpoles and adults of ranids and microhylids.

After surveying many areas in all the eight districts of Mizoram, the investigation has been devoted to study four species, *Euphlyctis cyanophlyctis* (earlier belonging to Ranidae but now to Dic平glossidae), *Hylarana nicobariensis* (Ranidae), *Kaloula pulchra* (Microhylidae) and *Microhyla berdmorei* (Microhylidae) which are found to be prevalent in Mizoram.

SPECIES NOMENCLATURES:

Euphlyctis cyanophlyctis was first described by Schneider (1799) as *Rana cyanophlyctis*. Earlier it was belonging under the family Ranidae. But recent taxonomic resolution adopted by Frost *et. al.* (2006) on the basis of molecular analysis, this species was transferred from the family Ranidae to the family

Dicoglossidae. However, since the thesis title was registered during the year 2004, it is still maintained under the family Ranidae in this thesis. The generic name *Euphlyctis* was introduced by Dubois (1992). *Hylarana nicobariensis* was originally described by Stoliczka in 1870 from Nicobar island as *Hylorana nicobariensis* which was later changed in to *Rana nicobariensis* by Boulenger in 1885. After many years, the present generic name, *Hylarana* is introduced by Che *et. al.* (2007) in place of *Rana*. *Kaloula pulchra* was described by Gray (1831) and the generic as well as specific name was still maintained till the date. *Microhyla berdmorei* was first described by Blyth (1856) as *Engystoma (?) berdmorei*, the current generic name, *Microhyla* was later introduced by Parker (1934).

Presentation of the thesis includes the following chapters which are as follows:

Chapter 1: Introduction

Chapter 2: Review of literature

Chapter 3: Materials and Methods

Chapter 4: Results

Chapter 5: Discussions

Conclusion

References

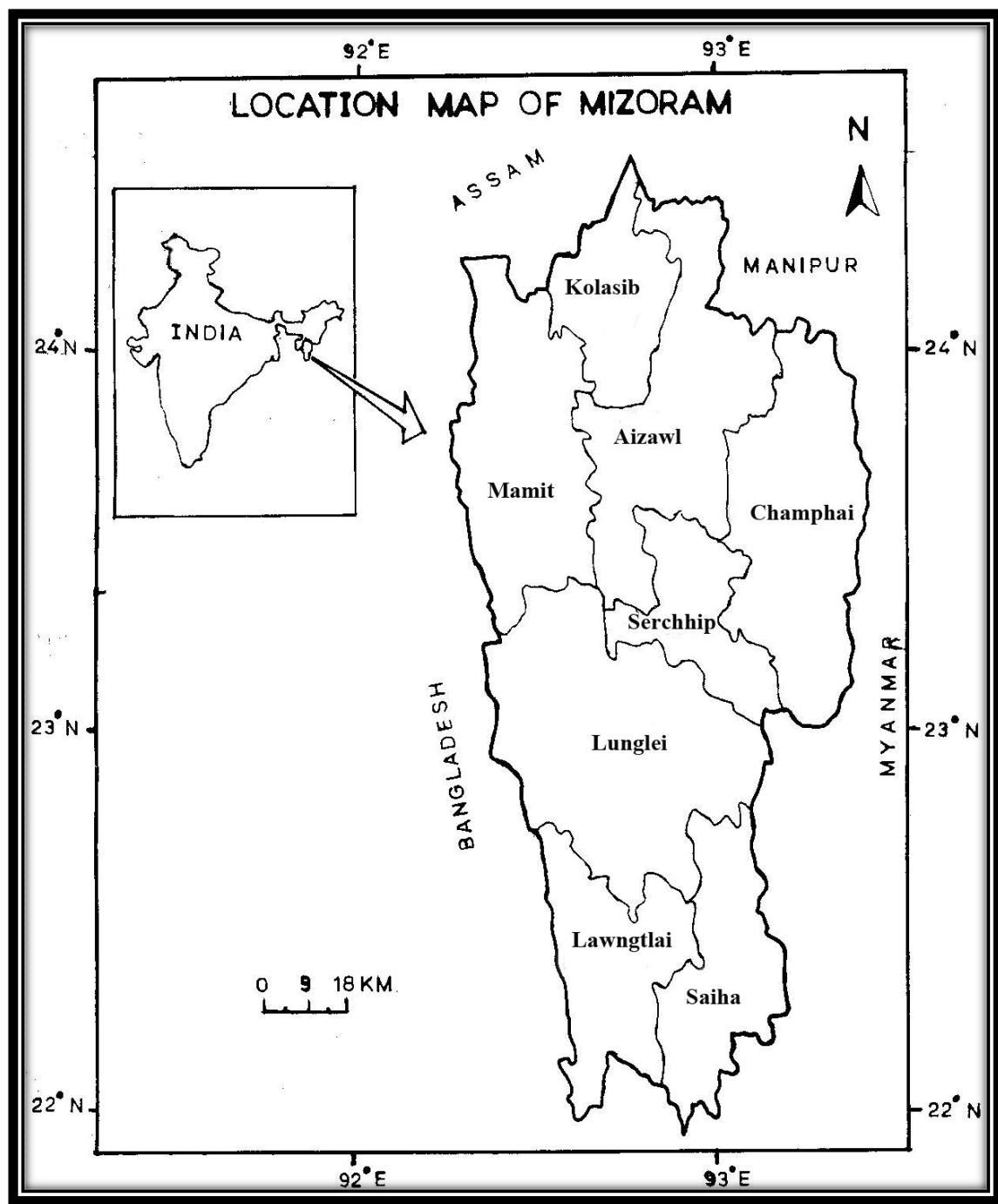


Fig. 1: Location of Mizoram



Fig .2.1: *Euphlyctis cyanophlyctis*

Fig. 2.2: *Hylarana nicobariensis*



Fig. 2.3: *Kaloula pulchra*



Fig. 2.4: *Microhyla berdmorei*

CHAPTER 2

REVIEW OF LITERATURE

`CHAPTER – 2

REVIEW OF LITERATURE

I. Distribution of Ranids and Microhylids:

Among the three major taxonomic components of amphibian diversity, the order Anura forms the largest living order (32 families, species, ca. 372 genera, 5227 species), these extend through all temperate and tropical lands except perpetually snow-capped mountain tops, waterless deserts, and some islands of the Pacific. They live in many environments, including grasslands, rain forests, alpine areas, and even deserts, although most species require freshwater habitats such as ponds, swamps, streams, or other wet environments for breeding. Caudata or Salamanders (10 families, 62 genera, 548 species) are largely Holarctic and Neotropical and are the best known amphibian group. Caecilians (6 families, 33 genera, 173 species) are found almost worldwide in tropical and warm temperate terrestrial, semi-aquatic and aquatic habitats. In altitude they range from sea level to over six thousands feet.

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), Amphibians of India and Sri Lanka (checklist and bibliography) by

Dutta (1997), Patterns of Distribution of amphibians: A global perspective by Duellman (1999), The Amphibian Tree of Life by Frost *et. al.* (2006) are some examples.

The existing literatures also provided many survey works on the distribution pattern of ranids and microhylids throughout the world. Savage (1973) provided a lengthy discussion of the distribution of anurans, in which he showed that the histories of the family group were intimately associated with the histories of the land masses that they occupied in the Mesozoic and Cenozoic. Duellman and Trueb (1994) mentioned that the major patterns of amphibian distribution at the familial and subfamilial levels can be explained best by vicariance biogeography. Some facets of peripheral distributions definitely are the results of dispersal, but these are relatively minor in comparison with the overall distribution patterns, except for some widespread genera such as *Bufo*, *Hyla* and *Rana*, in which both phenomena seem to have been important. However, Vences *et. al.* (2004) believed that the evidence from molecular clock calculations in Caribbean amphibians (e.g. Hedges *et. al.*, 1992) indicated that overseas dispersal may have played a major role in shaping the distribution of this fauna. Environmental variables are known to influence species distribution in many ways (e.g. Gascon 1991a; Leuven *et. al.*, 1986).

Crosswhite *et. al.* (1999) collected 886 individuals representing 38 species of reptiles and amphibians from the upland forests of the Ouachita Mountains, Arkansas. Woods *et. al.* (2004) surveyed the representative amphibians and reptiles of the Arid Southwest, United States. Loehle *et. al.* (2005) found that richness of amphibians and total herpetofauna, declined as stand ages increased from their studied on four watersheds in Arkansas. Werner *et. al.* (2006) studied on the status of two northern Leopard frog populations in Western Montana. Welsh *et. al.* (2007) conducted surveys and collections

on terrestrial forest amphibians from the 36 stands in Northwestern California, and they detected a total of 5,923 amphibians of 13 species.

Duellman (1966) recognized 6 major herpetological habitats (biociations) in Central America while studying ecological perspective on herpetofauna. Savage (1982) provided a detailed analysis of the Central American herpetofauna. Wilson and MacCrane (2003) reported 38 anuran species from the cloud forests of Honduras.

Brazil, with at least 751 species, has the greatest number of amphibians of any country on Earth, followed closely by Colombia. However, these results must be considered in relation to the level of survey effort (IUCN *et. al.*, 2006). Pearman (1997) addressed whether the habitat variation associated with logging agriculture is correlated with species richness and community structure in an assemblage of amphibians in the Tropical Wet forest life zone in Amazonian Ecuador by describing and evaluating 10 variables environmental variations and forest structure at 23.05 ha sites and sampled amphibians using nocturnal visual searches of transects. Duellman (1999) reported that the variation in amphibian diversity within the Amazon is related to altitude, topography, and rainfall, with historical biogeography and reproductive modes. In order to assess the main factors influencing its structure, Eterovick and Sazima (2000) studied an anuran community in a 1200 m high rocky meadow site at the Serra do Cipó, Minas Gerais, Brasil. Eterovick (2003) studied the composition of anuran assemblages in 16 streams at the Serra do Cipó, south-eastern Brazil, in which he found 26 anuran species. Diniz-Filho *et. al.* (2004) provided the linking of local processes with macroecological patterns in anurans from a local assemblage in Central Brazil. MacCulloch *et. al.* (2007) surveyed and turned up 14 amphibian and 8 reptile species belonging to 18 genera and 13 families from Mt. Roraima, Guiana Shield region, northeastern South America.

Allmon (1991) stated that amphibian diversity and abundance varies significantly among neotropics, Africa and Southeast Asia which has been attributed to differences in litter fall rates, mast fruiting, heterogeneity within regions, breeding habitat constraints, and geological history. African biodiversity has been extensively studied and, Daewood and Channing (2002) described a new cryptic species of African sand frog, *Tomopterna damarensis* (Anura: Ranidae), from Namibia. Poynton (2003) investigated the pattern of altitudinal species turnover shown by the rich fauna of frogs and toads in southeastern Tanzania. Glos (2003) analysed the amphibian fauna of the Kirindy forest in western Madagascar for four years and he reported 15 species out of four anuran families (Mantellidae, Ranidae, Hyperoliidae and Microhylidae), of which one species each from Ranidae and Microhylidae. Turner *et. al.* (2004) described a new moss frog species *Arthroleptella subvoce* (Ranidae) from the Groot Winterhoek Mountains in the Western Cape Province, South Africa.

Adnagulov *et. al.* (2000) studied the herpetofauna of some areas in the Khabarovsk Territory, Amurskaya Region, and Evreiskaya Autonomous Region in the Russian Far East. Zweifel (2000) worked on the Australopapuan microhylids and he recognized 35 species, of which 17 species (*Austrochaperina adamantina*, *A. aquilonia*, *A. archboldi*, *A. blumi*, *A. derongo*, *A. guttata*, *A. kosarek*, *A. novaebritanniae*, *A. parkeri*, *A. rivularis*, *A. yelaensis*, *Liophryne allisoni*, *L. rubra*, *L. similis*, *Oxydactyla alpestris*, *O. coggeri*, *O. stenodactyla*) were described as new and one was removed from synonymy.

Out of 5948 amphibian species all over the world, the size of the amphibian fauna of southern Asia can be estimated only roughly as >600 and probably <650 species. An exact count of known species cannot be made because species boundaries are so poorly understood in certain significant lineages. There also is uncertainty about the geographic

ranges of individual species, partly because of systematic problems but also because sampling of the fauna is seriously deficient in large areas. Southern Asia is essentially coterminous with the classic biogeographer's Oriental Region- the territory south of and including the southern flanks of the Himalayas, the Indian subcontinent east of the dry Indus Valley, Sri Lanka, southeastern Asia south of the Chinese border, the Greater and Lesser Sundas, Philipine Islands and Sulawesi. Present distributions also have been influenced by nonhistorical, ecological factors, such as seasonality of rainfall and topography (Inger, 1999). Indonesia can be predicted to be the richest country outside the Americas, but it is doubtful if even half of its species are yet known. It may end up with a level of diversity comparable with Brazil and Colombia (IUCN *et. al.*, 2006).

Yang (1991) reported that the genus *Amolops* includes about 20 species distributed from northeastern India and Nepal to south-central China to Peninsular Malaysia. Inger and Voris (1993) made a comparison on amphibian communities through time and from place to place Bornean forests by sampling 49 riparian frogs species along 18 streams at eight localities. Voris and Inger (1995) included 9 species (*Amolops phaeomerus*, *A. poecilus*, *Rana blythi*, *R. ibanorum*, *R. ingeri*, *R. kuhli*, *R. hosei*, *R. chalconota* and *R. signata*) of ranidae out of the total 19 frog species collected while investigating the abundance of frogs along streams in Bornean Forest. Inger and Lian (1996) prepared checklist of the frogs of Borneo. Zug *et. al.* (1998) surveyed on the herpetofauna of Chatthin Wildlife Sanctuary, Myanmar in which they had reported 8 species of Ranidae (*Occidozyga lima*, 2 *Rana limnocharis* complex, *Rana macrodactyla*, *Rana rugulosa*, *Rana tigerina* and *Tomopterna breviceps*) and 5 Microhylidae (*Glyphoglossus molossus*, *Kalophrynus interlineatus*, *Kaloula pulchra*, *Microhyla ornata* and *Microhyla* sp.) out of 16 species of amphibians. Inger *et. al.* (1999) reported new collections on the frogs from

Vietnam in which they had reported 15 ranids and 3 microhylids out of the total 100 species. Inger and Stuebing (1997), Manthey and Grossmann (1997), Inger (1999), Frost (2000) and, Iskandar and Colijn (2000) reported about 145 amphibian (mostly frog) and 250 reptile species from Indonesia, which Mittermeier (1988) identified it as one of the most important countries containing the world's "mega-diversity". Inger (1999) studied the distribution patterns of amphibians in Southern Asia and adjacent islands. Inger and Voris (2001) related the biogeographical relations of the frogs and snakes of Sundaland (Isthmus of Kra to Java and Sulawesi). Iskandar (2004) prepared annotated checklist with notes on ecological preferences and local utilization of the amphibians and reptiles of Malinau Region, Bulungan Research Forest, East Kalimantan. Khan (2004) annotated checklist of amphibians and reptiles of Pakistan. Veith *et. al.* (2004) reported 65 frog species and one caecilian species from the Kayan Mentarang National Park (East Kalimantan, Indonesia). Khan (2006) also prepared a book on amphibians and reptiles of Pakistan. Zainuddin (1999) reported a total of 18 species with 144 individuals of anurans occurred in Bario and vicinity, which only makes about 12.85% of the total species that occurred in Borneo. Das (2005), Das and Haas (2005a) and, Inger and Stuebing (2005) reported 41 species of ranids and 21 species of microhylids out of the total 154 species of anura in Borneo. Biswas and Pawar (2006) outlined an integrative framework for phylogenetic tests of distribution patterns in South Asia. Reza (2007) reported *Kalophrynus orangensis*, *Kaloula taprobanica* and *Sylvirana leptoglossa* from the country, Bangladesh. Inger and Chanard (1997) described a new species, *Rana archotaphus* and made a comment on *Rana livida*. Khan (1997) reported a new subspecies, *Euphlyctis cyanophlyctis* from Balochistan, Pakistan. Inger and Kottelat (1998) reported a new ranid frog of the genus *Amolops*, *Amolops cremnobatus* from Laos. Liu *et. al.* (2000)

discovered a new species of stream-breeding frog, *Amolops bellulus* from Western Yunnan, China. Khonsue and Thirakhupt (2001) reported the amphibian fauna of Thailand comprises of 130 species in 8 families and 3 orders. Diesmos *et. al.* (2002) described a new species of narrow-mouthed frog, *Kaloula walteri* from the volcanic mountains of southern Luzon Island (Mt. Banahao, Mt. Isarog, and Mt. Mayon) and adjacent Polillo Island, Philippines. Bain *et. al.* (2003) made taxonomic revisions on cryptic species of a cascade frog from Southeast Asia and described 6 new species (*Rana bacboensis*, *R. daorum*, *R. hmongorum*, *R. morafkai*, *R. banaorum* and *R. megatypanum*). Das and Haas (2003) described a new species *Kalophryalus eok* from the Kelabit Highlands of Sarawak at the border with Kalimantan. Orlov *et. al.* (2003) described a new ranid, *Rana trankieni* base on the morphological study of material collected in North Vietnam. Bain and Troung (2004a) studied on the herpetofaunal diversity of Ha Giang Province in Northeastern Vietnam, with descriptions of two new species, *Rana iriodes* and *Rana tabaca*. Bain and Troung (2004b) reported three new species of narrow-mouth frogs (Genus: *Microhyla*) from Indochina, with comments on *Microhyla annamensis* and *Microhyla palmipes*. Das *et. al.* (2004) described a new species of microhylid, *Calluella minuta*, from Sungai Relau, Taman Negara, Pahang State, Peninsular Malaysia. Leong and Lim (2004) reported *Rana siberu* from Peninsular Malaysia. Stuart *et. al.* (2005) discovered a new frog species, *Rana khalam* from 44 specimens from the mountains of southern Laos and central Vietnam. Bain and Stuart (2005) described a new species of cascade ranid, *Rana indepresa* 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. Inger and Iskandar (2005) prepared a list of anuran species from Padang area of West Sumatra with their distribution in altitudinal zones. Bain *et. al.*, (2006) described three new Indochinese species of cascade frogs (Amphibia: Ranidae) allied to *Rana archotaphus*. Bain and Stuart (2006) provided the new specimens of *Odorrana junlianensis* that represent the first provincial records from Yunnan and the first country records from Vietnam and Laos. Matsui and Nabhitabhata (2006) reported a new species, *Amolops panhai* from western to Peninsular Thailand. Matsui and Jaafar (2006) described a new species of cascade frog, *Rana monjerai* from west Malaysia. Orlov *et. al.* (2006) described a new frog species, *Rana gigatympana* of the family Ranidae (cascade frog from *Odorrana*-group) from central Vietnam. Matsui *et. al.* (2007) described a new species of frog, *Fejervarya sakishimensis* allied to *Fejervarya limnocharis* from the southern Ryukyus, Japan. Rao and Wilkinson (2007) described a new species *Amolops caelumnoctis* from a mountainous area of southern Yunnan Province, China.

Inger (1999) stated that the fauna of India/Sri Lanka is the most distinctive, with a number of endemic lineages (as opposed to species). Several recent publications have pointed out the discovery that many new species of frogs remain to be described in Sri Lanka (Dutta and Manamendra-Arachchi, 1996; Pethiyagoda and Manamendra-Arachchi, 1998; Meegaskumbura *et. al.*, 2002a&b; Pennisi, 2002; Bossuyt *et. al.*, 2004) and probably also in southern India, especially in the Western Ghats (Biju, 2002). If confirmed, these findings would much more than double the number of frog species in Sri Lanka, and increase significantly the number of amphibian species in India (Dubois, 2004). Indian amphibians, which are about 265 species in number (Das and Dutta, 2007) have a very high representation of endemics, with two major centres of distribution, north-east India

and the Western Ghats. Nearly sixty-three (63%) of the amphibians are endemic to India. Western Ghats is the richest region in India in terms of amphibian endemism. Ninety three taxa are endemic to this biogeographic region with two more taxa sharing their distribution with adjacent areas. In India during the last few decades several workers have contributed to distribution of amphibian fauna. Inger and Dutta (1996) gave an overview of the amphibian fauna of India. Das (1994) prepared a check-list of amphibians and reptiles of Andaman and Nicobar Islands. The situation in India is set to change dramatically with over 100 species in the process of description (IUCN *et. al.*, 2006). Das (1996b) reported *Limnonectes shompenorum*, a new species of ranid frog of the *Rana macrodon* complex from Great Nicobar. Das (1998a) described a new species *Rana charlesdarwini* from Mount Harriet National Park, South Andaman Island. Das (1998b) reported a new species, *Rana chitwanensis* from the Terai of Nepal. Sekar (1999) reported four new records and checklist of amphibians from Maharashtra. Abraham *et. al.* (2000) conducted a survey on amphibian fauna of Wayanad Wildlife Sanctuary, Kerala, in which he reported 30 species. Chanda *et. al.*, (2000) prepared a catalogue of amphibian types in the collection of the Zoological Survey of India. Krishnamurthy *et al.*, (2001) reported a new species of frog, *Nyctibatrachus hussaini* (Anura: Ranidae) from Western Ghats. Vasudevan *et. al.* (2001) reported the structure and composition of rainforest floor amphibian communities in Kalakad-Mundanthurai Tiger Reserve. Padhye and Ghate (2002) worked on the amphibian fauna of Maharashtra enlisting 43 species of amphibian. Padhye *et. al.* (2002) overviewed the amphibian fauna of Pune district which consisted of 31 species. Krishnamurthy (2003) gave amphibian assemblages in undisturbed and disturbed areas of Kudremukh National Park, central Western Ghats. Das *et. al.* (2003) reported notes on herpetofauna of Talchhapar Wildlife Sanctuary, Rajasthan. Siliwal *et. al.*

(2003) surveyed and reported 9 species of amphibian from Purna Wildlife Sanctuary, Dangs, Gujarat. Vyas *et. al.* (2003) recorded the occurrence of *Haplobatrachus crassus* from Gujarat. Vyas (2004) made a detailed survey on the herpetofauna of Vansda National Park, Gujarat. Das and Rathore (2004) carried out the study of the herpetofauna of Desert National Park, Rajasthan. Andrews *et. al.* (2005) surveyed the amphibian fauna of Kerala and described their distribution and status. Das and Kunte (2005) reported a new species, *Nyctribatrachus petraeus* (Anura: Ranidae) from Castle Rock, Karnataka state, South west India. Vasudevan *et. al.*, (2006) recorded a total of 2760 anurans from Ashambu Hills and 924 from Anamalai Hills belonging to 28 species of four families (Bufonidae, Microhylidae, Ranidae, and Rhacophoridae). Gaur and Pandey (2007) studied on the amphibian fauna of Thar desert from Rajasthan.

Northeastern India, which has a very high diversity among amphibians does not have many endemics within the Indian context because of the jagged political boundary of the country. However, all evidence suggests that Northeast India is a region very rich in amphibians as well as reptiles, and that much of this diversity still remains to be discovered (Pawar and Birand, 2001). Pawar (1999) stated that though North-east India, one of the biodiversity hotspots of the world, is one of the most beleaguered areas in terms of habitat degradation and loss today, little work has been done there in terms of herpetofaunal inventorying. A few decades ago, many workers had described new species and the occurrences of some Ranids and Microhylids in northeast India. Pillai and Chanda (1977) recorded two new species of frogs (Ranidae) from Khasi hills, India. Pillai and Chanda (1979) described the amphibian fauna of Khasi hills, Meghalaya. Pillai and Chanda (1981) reported the amphibian fauna of Garo hills, Meghalaya and described a new species of *Rana*. Chanda (1990) described a new frog *Rana mawlyndipi* (Ranidae)

from Khasi hills, Meghalaya, India. Chanda (1994) reported as many as 54 anuran species under 6 families and 18 genera in Northeast India. Kiyasetuo and Khare (1986) 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); 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). Ao *et. al.* (2006) provided the present distribution as well as redescription of holotype and morphology of adults and tadpoles of *Pterorana khare*. Chanda (1988) highlighted Amphibian (Anuran) species in Northeast India with their altitudinal distribution. Chanda (1994) reported the anuran (amphibian) fauna of north east India and in 1995 compiled the state fauna series 4 of anuran (amphibia) of Meghalaya (Chanda, 1995). Bordoloi and Borah (1999) gave the first record of *Hoplobatrachus crassus* from north eastern region in Assam and Arunachal Pradesh. Choudhury *et. al.* (1999) presented a range extension of *Uperodon globulosus* in Assam. Das and Chanda (2000) described a new species of the family Megophryidae, *Scutiger mokokchungensis* from Nagaland. Deuti *et. al.* (2000) rediscovered *Chirixalus simus* from Assam and West Bengal. Dey and Gupta (2002) reported *Kaloula pulchra* from Cachar district, Assam. Dutta *et. al.* (2000) reported the microhylid genus, *Kalophrynus* for the first time from India, and a new species, *Kalophrynus orangensis* was described from the Orang National Park, Assam in north-eastern India. Borah and Bordoloi (2001a) described nine new records of amphibian from Arunachal Pradesh, India. Borah and Bordoloi (2001b) rediscovered *Bufo macrotis* from Arunachal Pradesh. Choudhury *et. al.* (2001) studied the amphibian fauna of Kamrup district, Assam with

notes on their natural history. Deuti and Dutta (2002) made the first record of Boulenger's tree frog *Chirixalus vittatus* (Anura: Rhacophoridae) from Mizoram. Hooroo *et. al.* (2002) reported for the first time *Kaloula pulchra* from Cherrapunjee, East Khasi Hills district, Meghalaya. Ao *et. al.* (2003) presented the amphibian fauna of Nagaland with 19 new records from the state including 5 new records for India. Matthew and Sen (2003a) made rediscovery of *Micrixalus borealis* Annandale, 1912 (Ranidae: Anura: Amphibia) from Meghalaya. Das *et. al.* (2004) reported a new species of *Kaloula* (Anura: Microhylidae) from north eastern India. Grosselet *et. al.* (2004) reported *Microhyla heymonsi* for the first time from the mainland of India (Silchar, Assam) and studied its advertising calls. Sen (2004) gave further notes on state wise distribution of the amphibian fauna of northeast India. Sen and Matthew (2004) reported the occurrence of *Euphlyctis hexadactylus* (Amphibia: Anura: Ranidae) in north east India with notes on its morphological characters. Sailo *et. al.* (2005) recorded the occurrence of *Kaloula pulchra* from Mizoram. Hussain *et. al.* (2007) reported *Rana humeralis* from Assam and Arunachal Pradesh, and presented notes on its morphometry and natural history. Ningombam and Bordoloi (2007) carried out the amphibian fauna of Loktak lake, Manipur and recorded 10 new species for the state out of 25 species. Sengupta *et. al.* (2007) reported a new species, *Amolops assamensis* (Ranidae) from Assam. Pawar *et. al.* (2007) tested for congruence in complementarity between amphibians, reptiles and birds across seven tropical rainforest sites in the Eastern Himalaya and Indo-Burma global biodiversity hotspots. Das (2008) and Das *et. al.* (2009) reported 23 species of amphibian from Barail Wildlife Sanctuary, Assam. Ahmed *et. al.* (2009) prepared a photographic guide for amphibians and reptiles of northeast India, in which they reported 10 species under Microhylidae and 19 species under Ranidae from

this region. Sengupta *et. al.* (2010) reported a new species, *Leptolalax tamdil* (Megophryidae) from Mizoram.

II. Breeding behavior and Ecology of Ranids and microhylids:

Most anurans, like other amphibians, go through an aquatic stage as tadpoles and a terrestrial adult stage at some point in their lives. Typically, adults will return to the water to breed and deposit eggs. Duellman and Trueb (1994) highlighted two basic reproductive patterns among anurans. Most tropical and sub-tropical 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. However, 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, 1991a; Donnelly and Guyer, 1994).

Review of literature revealed that there are a number of studies on the reproductive behavior of ranids and microhylids in relation to mating calls, courtship, oviposition site, mode of reproduction, clutch size, sexual dimorphism and breeding cycle at the breeding site. Berry (1964) studied the breeding patterns of 7 species of anura (*Bufo melanostictus*, *Rhacophorus leucomystax*, *Rana limnocharis*, *Leptobrachium nigrops*, *Kaloula pulchra*, *Microhyla butleri* and *M. heymonsii*) representatives of all five anuran families occurring in Singapore. Species that breed in rivers and streams that are permanent bodies of water receive far less attention, even though they are commonly found in tropical and sub-tropical regions (Inger, 1966, 1969; Duellman, 1988). Up to

now, detailed information on the seasonal activity and reproduction of the stream species in tropical and sub-tropical regions is lacking. Kam *et. al.* (1998) studied the seasonal activity, reproduction, and diets of riparian anuran, *Rana swinhoana*, from a subtropical hardwood forest in Taiwan.

Daniels (1999) reported that amphibian studies in India had traditionally focussed on surveys and taxonomy. Until now very little is known on the breeding and development of microhylid frogs (Dutta *et. al.*, 1990-1991). However, publications of Morgan-Davies (1958), Inger and Frogner (1979), Khan *et. al.* (1979), Kirtisinghe (1957), Mallick and Mallick (1986), Mallick *et. al.* (1980), Mohanty Hejmadi *et. al.* (1977a, 1977b), Murthy (1968), Padhye and Ghate (1989), Sekar (1987) and Smith (1916) provide various information on the breeding, life history, development and tadpoles of several species of microhylid frogs from India and abroad. Hirschmann and Hödl (2006) studied on the visual signalling in *Phrynobatrachus krefftii* (Anura: Ranidae) at the Amani Nature Reserve, Usambara mountains, Tanzania.

The breeding behavior of ranids in India was observed by few workers, *Rana* (*Hoplobatrachus*) *tigerina* (McCann, 1932; Dutta and Mohanty-Hejmadi, 1976), *Rana* (*Euphlyctis*) *cyanophlyctis* (Mohanty-Hejmadi and Dutta, 1979; Mallick, 1988). Murthy (1968), Rao (1918) and Kirtisinghe (1957) provide some information on the breeding habits of *Ramanella variegata*. Dutta *et. al.* (1990-1991) gave information on the breeding and development of *Ramanella variegata* in Orissa, India. Kanamadi *et. al.* (1994a, 2002) studied on the advertisement call and breeding period of the frog, *Microhyla rubra* and *Kaloula pulchra*. Krishna *et. al.* (2004) reported on the breeding ecology of *Ramanella montana*, a rare and secretive microhylid, endemic to the Western Ghats, India.

Courtship and Mating calls: 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 contacts him; then he clasps the female. The most prominent mode of communication in anuran amphibians is, by far, the use of sound production. It 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). Male frogs and toads produce, species specific, breeding advertisement calls intended to attract gravid females. Females are known to respond positively to conspecific calls while responding indifferently to calls from other species (Littlejohn, 1965; Snyder and Jameson, 1965).

Reviews dealing with various aspects of vocalization in ranids and microhylids have been published. Heyer (1971) reported mating calls of some frogs from Thailand. Kuramoto (1987) studied on the advertisement calls of *Microhyla heymonsi* and *M. ornata*. Matsui *et. al.* (1993) described advertisement call characteristics of *Amolops chunganensis* from western China, *A. larutensis* from Peninsular Malaysia and *A. jerboa* from Borneo. Jehle and Arak (1998) described the vocal repertoire of the Asian cricket frog *Rana nicobariensis* for the first time. Given (1999) demonstrated the advertisement calls of male carpenter frogs (*Rana virgatipes*). Brooke *et. al.* (2000) examined the factors influencing acoustic displays in the tropical microhylid frog, *Cophixalus ornatus* from Australia. Wycherley *et. al.* (2001) developed new computer analytical techniques that aids bioacoustic separation of Norfolk pool frog, *Rana lessonae* populations. Bee (2002) studied on the socially mediated pitch alteration by territorial male bullfrogs, *Rana catesbeiana*. Lardner and Lakim (2002) showed the calling male Bornean tree-hole frogs

(*Metaphrynella sundana*) actively exploit the acoustic properties of cavities in tree trunks that are partially filled with water and which are primarily used as egg-deposition sites. Wycherley *et. al.* (2003) applied male advertisement call characteristics for the identification of introduced water frogs in Britain. Sun and Narins (2005) mentioned that response to anthropogenic sounds significantly decreased the calling rate of the three most acoustically active pondedge species (*Microhyla butleri*, *Rana nigrovittata* and *Kaloula pulchra*). 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. Ohler and Grosjean (2005) described and compared the advertisement call of *Kalophryalus interlineatus* from northern Vietnam and to that from a population of northern Thailand. The calls of 101 species of anuran amphibians (65.6%) known from Borneo were described by Sukumaran *et. al.* (2006). Kuramoto and Joshy (2006) made morphological and acoustic comparisons of three morphologically poorly defined *Microhyla* species, *Microhyla ornata*, *M. fissipes* and *M. okinavensis* on the basis of samples from south India, Taiwan and three Islands (Iriomote-jima, Okinawa-jima and Amami-oshima) of the Ryukyu Archipelago, Japan. Garietta and Martins (2009) presented new data on habitat, call, morphometry, eggs, and defensive behavior of a microhylid, *Arcovomer passarellii* based on a sample from Ubatuba, northeastern Sao Paulo state, Brazil Atlantic Forest. Sukumaran *et. al.* (2010) described the temporal and spectral properties of advertisement calls of Bornean anuran amphibians including *Microhyla borneensis* and *Hylarana signata*. Zainudin *et. al.* (2010) also described mating calls of five species of *Hylarana*, *H. baramica*, *H. glandulosa*, *H. signata*, *H. picturata* and *H. luctuosa* from Sarawak, Malaysia. Dehling (2010) studied on

the advertisement calls of two species of *Microhyla borneensis* and *M. petrigena* from Borneo.

In India, Kanamadi *et. al.* (1994b) presented the advertisement calls of *Rana tigerina* 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.* (1995) analysed the significance of female reciprocal call in certain Indian frogs. 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*. Kanamadi *et. al.* (1993a, 1994a, 2002) studied on the advertisement call and breeding period of the frog, *Polypedates maculatus*, *Microhyla rubra*, and *Kaloula pulchra*, respectively. Krishna *et. al.* (2004) studied on the breeding ecology including advertisement call of *Ramanella montana*. Acoustic communication studies in Western Ghat microhylids are limited to *Microhyla omata* (Hiremath, 1991), *M. rubra* (Kanamadi *et. al.*, 1994a), *Ramanella variegata* (Kanamadi *et. al.* 1993b), and *R. montana* (Kadadevaru *et. al.*, 1998). Giaretta and Martins (2009) presented new data on habitat, call, morphometry, eggs, and defensive behavior of *Arcovomer passarellii* based on a sample from Ubatuba, northeastern Sao Paulo state, Brazilian Atlantic Forest. In northeast India, analysis of anuran advertisement call limited to *Microhyla heymonsi* from Assam (Grosselet *et. al.*, 2004).

Oviposition site: Most species of frogs and toads rely upon water bodies for reproduction, and species vary considerably in the kinds of sites used for spawning (Crump 1974; Pough *et. al.* 2004). Crump (2000) reported that some anuran species have tadpoles that can develop successfully only in still water, whereas others are adapted to flowing-water systems. Suitable water bodies often are relatively scarce within the landscape, and the attributes likely to influence their suitability for oviposition may include factors, such as availability of vegetated calling sites close to the water's edge (Pough *et. al.*, 2004). Oviposition sites are a critical parameter of reproductive success in any species that does not move its young immediately after laying. Several studies indicate that the sites where eggs are oviposited can be influenced by the physical features of breeding sites as well as by the presence of predators, competitors and communal egg masses (Crump 1971, Maiorana 1976; Resetarits and Wilbur, 1989; Donnelly and Guyer, 1994; Holomuzki, 1995; Laurila and Aho, 1997). Various studies have suggested that female anurans select oviposition sites based on factors such as water depth (Herreid and Kinney, 1967; Metter, 1968; Zug and Zug, 1979; Petranka, 1984; Petranka and Sih, 1986), water temperature (Waldman, 1982; Crump, 1991), floods (Fukuyama and Kusano, 1992), or absence of predators (Peterson *et. al.*, 1992). Amphibian breeding-site selection is also described to be influenced by the combination of predators including fishes and amphibians (Resetarits and Wilbur, 1989; Kats and Sih, 1992; Murphy, 2003a), embryonic and larval densities of other amphibians (Resetarits and Wilbur, 1989; Murphy, 2003b), temperature (Seale, 1982), pond drying (Spieler and Linsenmair, 1997) and competition (Shepard, 2004). Therefore, Eterovick and Barros (2003) reported that the influencing role of adults in choosing mating sites limits tadpole distribution among different streams, as distribution of tadpoles and their temporal patterns of occurrence result from the spatial and temporal

distribution of reproductive effort by adult frogs, which can be influenced by many factors other than the ecological requirements of their larvae (Alford, 1999). Gilliespie *et. al.*, (2004) studied on the habitat use by stream-breeding frogs in south-east Sulawesi, with some preliminary observations on community organization. Adults can base their choices of oviposition sites on factors other than tadpole microhabitat availability, such as forest canopy cover (Werner and Glennemeier, 1999), presence of tadpole predators (Resetarits and Wilbur, 1991; Spieler and Linsenmair, 1997), and permanence and surface area of water bodies (Gascon, 1991a).

Mode of Reproduction: The diversity of reproductive modes in amphibians is higher than in any other vertebrate group (Caldwell, 1992). 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 (1994) 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 type of parental care, if any.” Brown and Alcala (1983) explicitly add larval nourishment to this list. Duellman and Trueb (1994) proposed three general modes (eggs aquatic, eggs terrestrial or arboreal, and eggs retained in oviducts), and divided these into 29 specific modes. In recent studies on ecology and natural history new reproductive modes have been described (e.g., Haddad and Höld, 1997; Haddad and Pombal Jr., 1998; Haddad and Sawaya, 2000; Prado *et. al.*, 2002). Further studies on the reproductive patterns of anurans have been described (Hodl, 1990; Kam *et. al.*, 1995; Biju, 2003; Haddad and Prado, 2005).

Morphometric measurement and Clutch size: Different in size among intraspecies was studied by various workers (Shine, 1987; Baker *et. al.*, 1990; Riha and Berven, 1991; Case

and Schwaner, 1993; Endler and Houde, 1995; Nussbaum and Wu, 1995; Partridge and French, 1996; Van Der Have and De Jong, 1996; Baez and Brown, 1997; Malhotra and Thorpe, 1997; Arnett and Gotelli, 1999; Schneider *et. al.*, 1999; Wiens *et. al.*, 1999; Avise, 2000; Castellano *et. al.*, 2000; Demetrius, 2000; Storz *et. al.*, 2001; Schäuble, 2004; Silva and Rossa-Ferreira, 2010).

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). Matsui and Ota (1984) provided the parameters of fecundity in *Microhyla ornata* from the Yaeyama group of the Ryukyu Archipelago. Kusano and Hayashi (2002) provided female size-specific clutch parameters of two closely related stream-breeding frogs, *Rana sakuraii* and *R. tagoi tagoi* emphasizing female size-dependent and size dependent egg sizes.

Globally, investigation on the variation of clutch size of ranids and microhylids have been reported by various workers (e.g. Pope, 1931; Inger and Bacon Jr., 1968; Matsui and Ota, 1984; Ponsero and Joly, 1998; Loman, 2001; Kusano and Hayashi, 2002; Schleich and Kästle, 2002). In India, similar studies from have been reported by Morgan-Davies (1958), Dutta and Mohanty-Hejmadi (1976), Dutta *et. al.*, (1990-91) and Krishna *et. al.*, (2004), etc.

Sexual Dimorphism: Sexual dimorphism (SD) is a widespread phenomenon. In numerous species, pronounced differences between the sexes are found in various characteristics of morphology (body size and shape), coloration, ornaments, etc. (eg. Darwin, 1871; Anderson, 1994; Halliday and Tejedo, 1995). In amphibians in general, sexual dimorphism includes a diverse array of characteristics – size and shape differences, dorsal crests,

mating calls, etc. (Duellman and Trueb, 1994). In a review of published data, Shine (1979) estimated that females are larger than males in approximately 61% of urodeles (of 79 species reviewed) and 90% of 589 anuran species reviewed. Few workers have described sexual dimorphism in ranids (Liu, 1950; Boulenger, 1920; Inger *et. al.*, 1984; Kam *et. al.*, 1998; Krishnamurthy and Shakuntala, 1999; Vasudevan, 2003) and microhylids (Zweifel, 2000; Kuramoto and Joshy, 2006; Giaretta and Martins, 2009).

Breeding cycle: The annual reproductive pattern of amphibian has been studied extensively from the temperate and subtropical regions, and the physiological basis for their cyclic reproductive behavior has been dealt with (Inger and Bacon Jr., 1968; Oordt, 1960). The seasonal breeders are found in areas where the variation in the length of day may be insignificant but other environmental factors, like temperature, relative humidity and rainfall fluctuate markedly not only during different months but also from place to place. Three major environmental factors have been implicated in the regulation of the amphibian breeding cycles: rainfall, photoperiod, and temperature (Lofts, 1974). Annual reproductive cycle and seasonal activity have been studied in anurans (Gopalakrishnan and Rajasekarasetty, 1978; Huang *et. al.*, 1996; Huang *et. al.*, 1997; Ao and Bordoloi, 2000, etc.). Literature on breeding activity for various species from these two regions show that breeding activity is governed by either a single factor – temperature (Briggs, 1987; Fukuyama and Kusano, 1992), rainfall (Ritke *et. al.*, 1992; Donnelly and Guyer, 1994), moisture (Doreas and Foltz, 1991; Moreira and Barretto, 1997), wind (Robertson, 1986) or a combination (Lizana *et. al.*, 1994; Moreira and Barretto, 1997). Haddad and Sazima, (1992), and Duellman and Trueb (1994) reported that rain is the primary extrinsic factor affecting the time of the breeding activity for most tropical and subtropical anuran species although rainfall is associated with the breeding in some species, it seems to have less

influence once the breeding starts (Okuno, 1985). 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, 1994; Beebee, 1995). Diaz-Paez and Ortiz (2001) stated that reproductive patterns are correlated with prevailing climatic conditions. Changes in temperature and photoperiod stimulate gametogenesis, and establish continuous or discontinuous cycles (Jorgensen, 1992). 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 and 1984; Jorgensen *et. al.*, 1978; Jorgensen *et. al.*, 1986; Rastogi *et. al.*, 1976; Rastogi *et. al.*, 1983a&b). Species that inhabit tropical areas, with a constantly warm and humid climate, exhibit continuous or potentially continuous cycles (Church, 1960; Inger and Greenber, 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).

A large number of amphibian species shows annual reproductive cycles dependent on seasonal changes in environmental factors. In amphibia, as in other vertebrate groups, the internal hypophysio-gonadal rhythm is under the mark influence of a variety of external factor (Gopalakrishnan and Rajasekarasetty, 1978). Studies on seasonal changes in the testicular histology, and effects of photoperiod and temperature on seasonal spermatogenic cycles have been reported (Saidapur and Nadkarni, 1975b; Gopalakrishnan and Rajasekarasetty, 1978; Chavadej *et. al.*, 2000), and effects of progesterone on the testis (Kasinathan and Basu, 1975), thumb pad and pars distalis (Yajurvedi and Hooli, 1983), effects of estrogens on spermatogenesis and leydig cells (Saidapur and Nadkarni, 1975a; Rastogi and Iela, 1980; Kanamadi and Saidapur, 1982).

Emerson (1997) studied on the relation between testis size and competition, and its variation in some frogs. Structure of the testis, its changes during development and seasonal variation were also studied on *Rana (Haplobatrachus) tigerinus* (Sretarugsa *et. al.*, 1997) and *Rana catesbeiana* (Chavadej *et. al.*, 2000)

One of the most remarkable characteristics of amphibians is the change of ovarian cyclicity in correlation with the variation in environmental or seasonal conditions (Kanamadi and Saidapur, 1982), especially the seasonal climatic cycle of temperature and precipitation. Oogenesis in ovaries and classification of developing oocytes of anurans has been studied by many investigators (Duryee, 1950; Kemp, 1953; Grant, 1953; Wartenberg and Gusek, 1960; Balinski and Devis, 1963; Hoque and Saidapur, 1994). Further, the mechanism regulating the ovarian cycle, effect of photoperiod and temperature and seasonal variations in ovarian cycle in anurans have also been studied (Horseman *et. al.*, 1978; Rastogi *et. al.*, 1983b; Pancharatna and Saidapur, 1990; Saidapur, 1989; Whittier and Crews 1987; Wingfield and Kenagy, 1991; Saidapur and Hoque, 1995; Pawar and Pancharatna, 1999; Sretarugsa *et. al.*, 2001).

III. Development and metamorphosis:

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). Detailed descriptions of the ontogeny of embryonic, larval, metamorphic and post embryonic frogs (Anura) are important for understanding the general pattern(s) of development of representative taxa and, by comparisons, of different patterns, for resolving questions

about systematic relationships. For anurans, staging tables are a condensed way of describing ontogenetic changes (Hall *et. al.*, 1997). Therefore studies on the successive ontogenetic stages to record the normal developmental table are important in understanding the ecology of a species and for planning conservation measures. Amphibian development has been investigated extensively by many embryologists, who have taken advantage of the development of relatively large external eggs for both descriptive and experimental studies (Rugh, 1951; Harrison, 1969). The most comprehensive treatment of amphibian development within a broad biological context is the work of Salthe and Mecham (1974). During the larval period, amphibians, anurans in particular, exhibit a series of dramatic morphological changes (e.g., tail formation, perforation and closure to the spiracle, limb formation, tail reduction). 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. Duellman and Trueb (1994) defined metamorphosis as a series of abrupt post-embryonic changes involving structural, physiological, biochemical, and behavioral transformations, while Shi (2000) defined it as a post-embryonic period of profound morphological changes by which the animal alters its mode of living. 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. Anuran metamorphosis is separated into three specific periods - pre-metamorphosis, pro-metamorphosis and metamorphic climax (Etkin, 1964, 1968; Dodd and Dodd 1976). Amphibian metamorphosis has been investigated thoroughly by several workers (Etkin and Gilbert, 1968; Dodd and Dodd, 1976; Gilbert and Frieden, 1981; Fox, 1983; Shi, 2000).

Fox (1983) indicated that of the few thousand known species of anurans, there are complete staging tables (fertilized eggs through the completion of metamorphosis) for fewer than 20 species. Duellman and Trueb (1994) suggested that complete tables of development are necessary for accurate comparison of developmental stages in different organisms. Hence, tables of normal stages of development have been worked out for a number of species by several workers. The anuran development was described thoroughly by Rugh (1951), who also conducted experimental embryological work on amphibians in detail (Rugh, 1962). Many different methods have been developed to stage anurans during development, especially during metamorphosis (Just *et. al.*, 1981). Most of the tables that have been constructed are for species contained in the genera *Rana* and *Bufo*. Twenty-five prefeeding stages of *Rana pipiens* was tabulated by Shumway (1940). 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). Indeed, tables of developmental stages that describe morphological changes during the larval period have been proposed for quite a few amphibian taxa (see Gosner, 1960; Nieuwkoop and Faber, 1967; Fox, 1983; Iwasawa and Futagami, 1992; McDiarmid and Altig, 1999 and other works cited there in). Shi (2000) in the book *amphibian metamorphosis: from morphology to molecular biology* studied in detail on amphibian metamorphosis.

Yuan (1950) provided notes on the eggs, tadpoles and growth of *Rana longicrus* Stejneger from Taiwan. Earlier, complete tables of normal development are not yet available for any member of Microhylidae (Fox, 1983; McDiarmid and Altig, 1999). For the species *Microhyla ornata*, few previous studies yielded data on some aspects, such as size at metamorphosis (Maeda and Matsui, 1989; Dash and Dei, 1998) and larval

morphology at certain developmental stages (Chou and Lin, 1997; Khan, 2000; Schleich and Kästle, 2002). With respect to its embryonic and larval development, Liu *et. al.* (1996) provided a brief description on the period from fertilization to spiracle completion on the basis of materials from China. However, Shimizu and Ota (2003) studied the normal development of *Microhyla ornata*, and gave, for the first time a description of the complete embryonic and larval stages for the Microhylid frogs.

Recently, similar studies have also been conducted on the life history and metamorphosis of some ranids and microhylids from India. Although Rao (1915) and Ferguson (1904) have described a few larvae of the Indian burrowing frog *Rana breviceps*, there is considerable disagreement between the two regarding characteristics. However, Mohanty-Hejmadi *et. al.* (1979) 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, 1917; 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, 1979). Although Ferguson (1904) has given a description of the marbled balloon frog, *Uperodon systema*, Mohanty-Hejmadi *et. al.* (1979) demonstrated the life history and detailed characteristics of the larva of this species. Twenty five developmental stages of a microhylid, *Ramanella variegata* were described by Dutta *et. al.*, (1990-91).

Physico-chemical factors: The role of temperature in the biology of amphibians has received considerable attention since Moore (1939, 1942) showed the importance of temperature in the ecology and evolution of members of the genus *Rana* in eastern North

America. Studies using amphibian eggs show a correlation between embryonic temperature adaptations (developmental rate and temperature tolerance) and breeding season temperatures, or breeding habits and geographic distribution of the species (Moore, 1949; Volpe, 1957; Ruibal, 1962; Brown, 1967). The role of temperature on growth and development of tadpoles was demonstrated by other workers (Harkey and Semlitsch, 1988; Rome *et. al.*, 1992). Gibbs and Karraker (2006) reported that amphibians are of particular interest because environmental temperatures exert strong control over all aspects of their biology. Nie *et. al.* (1999) studied dissolved oxygen, temperature and habitat selection by bullfrog (*Rana catesbeiana*). Effects of higher temperature on the growth rates of anurans was studied by several workers (Gosner and Black, 1955; Straw, 1958; Hadfield, 1966; Brown, 1969; Lillywhite, 1970; Smith, 1976; Browne and Edwards, 2003). Growth in amphibians is primarily determined by temperature, water, and prey availability (Jorgensen 1992; Alvarez and Nicieza, 2002; Merila *et. al.*, 2004). Food availability has been demonstrated to affect growth rates and reproductive traits in post-metamorphic amphibians (Scott and Fore 1995).

Acidification of habitat is thought to have a major impact on amphibians and the structure of their populations. Gosner and Black (1957) studied on the effects of acidity on the development and hatching of New Jersey frogs. Several experimental studies were conducted to reveal the effects of acid water on embryos and larvae of different amphibian species (Andren *et. al.*, 1988, 1989; Rowe *et. al.*, 1992). Negative influence of low pH on embryonic development and hatching success in amphibians has been verified by many studies (Brunstrom, 1977; Pierce *et. al.* 1984; Clark and Hall, 1985; Dale *et. al.*, 1985; Petranka *et. al.*, 1982; Andren *et. al.*, 1988). Pierce *et. al.* (1984), Freda and Dunson (1985a), and Freda (1986) found that amphibian embryos are the most sensitive to low pH,

and tolerance increases with larval age. Pough (1976), and Freda and Dunson (1985b) reported that embryos exposed to extremely low pH arrests development. Rowe and Freda (2000) studied on the effects of acidification on amphibians at multiple levels of biological organization. Low pH also has been shown to decrease growth and development rates or to delay metamorphosis in *Rana temporaria* (Cummins, 1986b and 1989; Beattie and Taylor-Jones, 1992) and *Rana sylvatica* (Sadinski and Dunson, 1992; Rowe *et. al.*, 1992; Horne and Dunson, 1995). Pahkala *et. al.* (2001) found that survival probability and hatchling size were reduced by low pH in the moor frog *Rana arvalis*. Field investigations 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). Glos *et. al.*, (2003) demonstrated geographic variation in pH tolerance of two populations of the European common frog. Karraker (2007) studied on the effects of saline solution on the embryonic and larval *Rana clamitans*. Kuramoto (1975) studied the oxygen consumption of embryos of *Microhyla ornata* from Iriomotejima and noted that this species, like *Hyla japonica* and *Rana (Fejervarya) limnocharis*, apparently adapts to waters of high temperatures with only a little oxygen. Joshi and Mohinuddin (2003) studied the effect of red light in metamorphosing tadpoles of *Rana cyanophlyctis*. Gramapurohit *et. al.* (2004) studied on the relative influence of kinship and density on metamorphic traits of *Tomopterna breviceps*. Loman (1999) studied the influence of temperature on energy cost and timing of embryonic and larval development in a number of anuran species.

Larval morphology: Review of literature reveals that there are few descriptions on the larval morphology of the tadpoles of ranids and microhylids. Schijfssma (1932) reported on some tadpoles of Java. Berry (1972) described the undescribed and little known tadpoles

from West Malaysia. Heyer (1973) provided a brief description on 17 species of anuran tadpoles collected from Thailand. Kuramoto *et. al.* (1984) described on the larval morphology of Taiwanese brown frogs, *Rana longicrus* and *R. sauteri*. Inger (1985) described tadpoles of the forested regions of Borneo. Inger and Tan (1990) described tadpoles of ranid, *Staurois natator* from Borneo. Lim and Ng (1991) described the larval morphology and breeding habits of *Kalophryalus pleurostigma*. Leong and Chou (1999) constructed a key for identification of 22 different larval types (including 9 from Ranidae and 5 from Microhylidae) collected from Singapore, in which they focused on externally observable characteristics eg. position of spiracle, presence/ absence of marginal papillae, position of anal tube. Khan (2003a&b) studied on the larval hyobranchial skeleton of five anuran species and their ecological correlates from Pakistan. Inthara *et. al.* (2005) described the mouth part structure and distribution of 44 species of tadpoles from Thailand. Larval morphology of *Rana (Clinotarsus) alticola* was described from the northeastern India (Annandale, 1912; Sahu and Khare, 1983), peninsular Thailand (Smith, 1924), and Phang Nga Province and Thailand (Grosjean *et. al.*, 2003). Gregoire (2005) prepared a guide book for the tadpoles (including 7 ranids and 1 microhylid) of the southeastern United States coastal plain. Ming (2005) prepared larval systematic of the Peninsular Malaysian ranidae on the basis of 28 species. Altig (2007) summarized a primer for the morphology of anuran tadpoles. Rodrigues *et. al.* (2008) described the tadpole of *Chiasmocleis hudsoni* (Microhylidae) in Central Amazonia, Brazil. Hendrix *et. al.* (2008) described the tadpole of the Narrow-mouthed Frog *Microhyla fissipes* from Vietnam. Davis *et. al.* (2008) developed a fast, non-invasive method of measuring growth in tadpoles using image analysis.

In total, 118 species of amphibians known from India now have known tadpoles, representing 44.5% of the 265 known Indian amphibian species (Das and Dutta, 2007). This compares unfavourably with the Bornean– 55.6% (Das and Haas, 2005b) and Peninsular Malaysian– 76.1% (Leong, 2002) faunas, particularly since additional larval forms have been described or have been since recognised since these two reviews were published (e.g., Leong, 2004; Leong and Lim, 2003; Haas *et al.*, 2006; Inger *et al.*, 2006). Some descriptions on the tadpoles (ranids and microhylids) of India includes *Rana (Nasirana) alticola* (Annandale, 1912; Sahu and Khare, 1983). *Rana temporaria* (Hiragond and Saidapur, 1999), *Indirana beddomii* (Kuramoto and Joshy, 2002), *Microhyla ornata* (Dey and Gupta, 2002).

IV. Food and feeding in relation to oral structures and intestines:

McDiarmid and Altig (1999) stated that understanding morphology, especially that of the oral disc, is crucial to comprehending the feeding ecology of tadpoles and therefore likely is the foremost factor in interpreting most, perhaps all, aspects of their biology. To achieve access to different profitable nutritional resources, various lifecycle strategies have been evolved among anurans. The complex lifecycle of anurans is especially spectacular, among other reasons, because of the change from the aquatic to the terrestrial mode of life which is a shift in nutrition from a mostly herbivorous to a fully carnivorous diet (Feder and Burggren, 1992).

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. Altig and Johnston (1989), and (Pryor and Bjorndal, 2005a&b) mentioned that tadpoles are often overlooked and understudied relative to other consumer groups such as fishes and macroinvertebrates in freshwater ecosystems, and the true

trophic status of many tadpole species remains unknown. In addition to this, according to Altig *et. al.* (2007), basic information that is central to understanding their ecological significance and thus the consequences of their loss. In anuran tadpoles specialized parts of the bucco-pharyngeal passage take part in filtering the particulate suspension as water current passes through it (Wassersug, 1975; Seale and Wassersug, 1979; Wassersug and Hoff, 1979; Viertel, 1987; Sanderson and Wassersug 1989; Khan, 1991, 1999; Khan and Mufti, 1994). The morphology of the oral and bucco-pharyngeal structures of anuran tadpole species reflects their dietary preferences (Wassersug 1980; Inger, 1985; Altig and Johnston, 1989; Khan 1991).

Literature surveys revealed that oral morphology and feeding habits of tadpoles have been described in numerous species of Ranids and Microhylids. Many anuran larvae are considered primarily herbivorous, feeding on an impressive diversity of algal species (Farlowe, 1928; Savage, 1952; Kamat, 1962; Jennis, 1967; Hendricks, 1973; Seale and Beckvar, 1980; Diaz-Paniagua, 1985; Johnston, 1991; Hoff *et. al.*, 1999; Peterson and Boulton, 1999; Pryor, 2003 etc.). Studies of gut contents sometimes indicate interespecific and site differences in diet of anuran larvae (Heyer, 1976; Waringer-Loschenkohl, 1988; Skelly, 1995). Heyer (1973) and Inger (1986) examined the gut contents of tadpoles from Thailand and Borneo rainforests, respectively, to associate diet with microhabitat and recognized different modes of feeding. Wassersug (1976) gave an introduction to certain morphological features in the mouths of tadpoles and also gave a comparative investigation on the evolution of anuran larvae and the systematics of frogs. Khan and Malik (1987a) studied the buccopharyngeal morphology of tadpole larvae of *Rana hazarensis*, and its torrentocole adaptations. Khan and Mufti (1994) described in detail on the circum-oral region of Pakistani tadpoles and its morphology is correlated to

the feeding ecology of each species of tadpole and observation of mode of feeding of each species of tadpole was also recorded. In detailed field experiments, Kupferberg (1997a&b) demonstrated that certain algae diets produced better tadpole growth and faster metamorphosis than others, for several species of larval anurans. Seale and Beckvar (1980) compared the ability of *Hyla crucifer*, *Bufo americanus*, *B. woodhousei fowleri*, *Rana catesbeiana*, and *R. sylvatica* tadpoles to ingest suspended blue-green algae. Khan (1999) studied on the food particle retrieval in five amphibian tadpoles, namely *Bufo stomaticus*, *Euphlyctis cyanophlyctis*, *Hoplobatrachus tigerinus*, *Limnonectes limnocharis*, *L. sydhadrensis* and *Microhyla ornata*. Wassersug and Yamashita (2001) studied feeding kinematics in anuran larvae with a typical oral disc. Vences *et. al.* (2002) related environmental variables and the morphology of the common frog, *Rana temporaria*. Khan (2003a) studied on the external morphology including oral structure of *Euphlyctis cyanophlyctis* tadpole with notes on its feeding ecology. Grosjean *et. al.* (2004) described the evolutionary significance of oral morphology in the carnivorous tadpoles of tiger frogs, genus *Hoplobatrachus* (Ranidae). Altig (2006) discussed on the origin and evolution of the oral apparatus of anuran tadpoles. Echeverria *et. al.* (2007) studied on the diet of tadpoles from a pond in Iguazu National Park, Argentina.

Mohanty-Hejmadi *et. al.* (1979) and Mohanty-Hejmadi and Acharya (1979) observed on food habits of some species of Indian frogs. Review on the literatures revealed that many workers investigated the diets of ranids e.g. *Rana pipiens* (Drake, 1914), *Rana catesbeiana* (Brooks, 1959), *Rana clamitans* (Jennsen and Klimstra, 1966), *Rana temporaria* (Blackith and Speight, 1974), *Rana cyanophlyctis* (Battish and Sandhu, 1988), *Rana longicrus* (Kam *et. al.* 1995), *Rana swinhoana* (Kam *et. al.*, 1998), etc. Santos *et. al.* (2004) made the first report on feeding habits of anurans in rainforest

fragments in Northeastern Brazil. Kam *et. al.* (1998) studied seasonal activity, reproduction, and diet of a riparian frog (*Rana swinhoana*) from a subtropical forest in Taiwan. For the species *Microhyla ornata*, few previous studies yielded data on some aspects, such as buccopharyngeal morphology and feeding ecology (Khan, 2000). Pryor (2003) investigated on the growth rates and digestive abilities of larval bull frogs, *Rana catesbeiana* fed monospecific algal diets. Khan (2004b) reported the breeding habitats and feeding ecology of *Haplobatrachus tigrinus*. Iwai and Kagaya (2005b) studied the difference in larval food habit of two Japanese *Rana* species, *Rana japonica* and *Rana ornativentris*.

Similarly, in India few workers reported the diets of tadpoles and adults with a few descriptions on the oral morphology. Rao (1933) observed on the feeding behavior and oral structure of Engystomatid tadpoles. Mohanty-Hejmadi *et. al.* (1979) 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. Study of food of tadpoles in northeast India was earlier done by Sahu and Khare (1983) on *Rana alticola* tadpoles in Meghalaya. Das and Coe (1994) demonstrated the dental morphology and diet in anuran amphibians from south India. Das (1994) demonstrated the internal oral morphology of some ranid and microhylid larvae from south India with the help of a scanning electron microscopic study. Das (1996a) studied on the folivory and seasonal changes in diet in *Rana hexadactyla* from Tamil Nadu. The first study on food habit of tadpoles in Arunachal Pradesh was accomplished by Sinha *et. al.* (2000).

Many workers have traditionally viewed tadpoles as being herbivores that occupy lower trophic levels in aquatic communities. Their highly efficient gill filters and mucus entrapment mechanisms (Kenny, 1969; Wassersug, 1972) provide tadpoles with

rapid ingestion rates of suspended algae (Seale and Beckvar, 1980), whereas their serrated, keratinized jaw sheaths and labial teeth – which bear a striking resemblance to the radulae of herbivorous snails (Stenick and Watling, 1982) – allow the same tadpoles to graze periphyton effectively (Wassersug, 1984; Kupferberg, 1997a; Altig and McDiarmid, 1999). Such morphological specializations, coupled with high food intake (Wassersug, 1984) and a high population densities (e.g. thousands of individuals m⁻²; Alford, 1986) suggest that tadpoles have considerable impact on nutrient cycling and primary production in freshwater ecosystems (Pryor, 2003). Many workers demonstrated the oral structures of ranids and microhylids larvae. Khan (1982) provided a key for the identification of amphibian tadpoles from the plains of Pakistan with a brief description on oral disc and ecology. Sekar (1990) provided the food composition of *Rana curtipes*. Wassersug and Yamashita (2001) studied feeding kinematics in anuran larvae with a typical oral disc. Rachowicz (2002) studied on the mouthpart pigmentation in *Rana mucosa* due to seasonal changes without chitridiomysis.

The alimentary tract of anuran larvae has been studied by numerous workers (e.g., Barrington, 1946; Griffiths, 1961; Fox, 1984; McAvoy and Dixon, 1977; Dauca and Hourdry, 1985; Rada and Bello, 1988; Viertel and Richter, 1999; Akiyoshi *et. al.*, 2002; Nakajima *et. al.*, 2005). Bonneville (1963) investigated fine structural changes in the intestinal epithelium of the bullfrog during metamorphosis. Marshall and Dixon (1978) reported on the cell specialization in the epithelium of the small intestine of feeding *Xenopus laevis* tadpoles. The general morphological pattern is that of an almost undifferentiated tube, consisting of an esophagus, a gastric region, and a long coiled intestine (Hourdry and Beaumont, 1985). Michel de Cerasoulo and Terán (1991) correlated morphological features with different diets, recording the main differences in

the cellular physiology. At a macroscopic level, longer digestive tracts are correlated with a mainly herbivorous diet, whereas shorter ones are associated with carnivorous diets (Noble, 1954; Altig and Kelly, 1974; Villee *et. al.*, 1987; in Martin *et. al.*, 1997). Metamorphic shortening of the alimentary tract of larvae was studied on different anurans e.g. *Rana pipiens* (Janes, 1934), *Alytes obstetricians* (Dauca and Hourdry, 1985), *Rana catesbeiana* (Carver and Frieden, 1977; Pretty *et. al.*, 1995). Wilczyńska *et. al.* (2004) studied on the changes in the structure of the alimentary canal at selected stages of development of *Phrynohyas resinifictrix*. Schreiber *et. al.*, (2005) demonstrated on the remodeling of the intestine during metamorphosis of *Xenopus laevis*.

CHAPTER 3

MATERIALS AND METHODS

CHAPTER – 3

MATERIALS AND METHODS

I. Distribution of ranid and microhylid anurans in Mizoram:

To know the distribution of ranids and microhylids prevalent in Mizoram, survey was conducted from the year 2004 and general observations of frogs were made throughout the forest, streams, rivers and other aquatic habitats, such as lakes, ponds, rice paddies, ditches and pools in plantations. The location (latitude/longitude) and elevation of the surveyed areas were determined with the help of Garmin (etrex) Global Positioning System (GPS). Standard survey techniques for amphibians include anuran calling surveys, egg mass surveys, larval surveys, and visual searches for adults. Specimens from different localities of Mizoram were collected. After making careful observations, the collected specimens were identified, morphometric measurements were taken and classified using the published guides. A set of voucher specimens were also sent to the Zoological Survey of India (ZSI), Eastern Regional Station for identification. The surveyed areas represents all the eight districts, Aizawl district, Champhai district, Kolasib district, Lawngtlai district, Lunglei district, Mamit district, Saiha district and Serchhip district (Fig. 3.1).

(1.) Study animals: After surveying different parts of Mizoram, four species which are prevalent in Mizoram, *Euphlyctis cyanophlyctis* (earlier belonging to Ranidae but now to Dic平glossidae), *Hylarana nicobariensis* (Ranidae), *Kaloula pulchra* (Microhylidae) and *Microhyla berdmorei* (Microhylidae) are selected to study their

ecology, breeding behaviour and their development. A brief account of these four frog species are given below:

(i.) *Euphlyctis cyanophlyctis* (Schneider, 1799): Indian skipping frog ranges throughout much of South Asia including southern Afghanistan and Sri Lanka. It is also present in southeastern Iran. It is the only wide ranging thoroughly aquatic, littoral and most frequent frog found resident in almost every water body throughout its range. Its unique habit of skittering on water surface fascinates equally an observer (Fig. 2.1). Although it is reported from Mizoram, there is no information on the biology of this species in Mizoram.

(ii.) *Hylarana nicobariensis* (Stoliczka, 1870): *Hylarana nicobariensis* is also known as Nicobar island frog, Cricket frog, Nicobarese frog, Nicobar frog and Nicobar cricket frog. In Mizoram, this species is one of the most common frogs and found primarily in fast-flowing streams and rivers of moderate to steep gradient and their adjacent water bodies like, lakes, ponds and paddy fields (Fig. 2.2).

(iii.) *Kaloula pulchra* Gray, 1831: The Malaysian painted frog is also known as the painted or Asian bullfrog, chubby frog, rice frog and bubble frog. It is native to Southeast Asia. They occur naturally in a wide variety of habitats, from populated villages, to rice fields, to leaf-covered forest floors. *Kaloula pulchra* is different from most frogs, which tend to stay away from towns and other places where people have moved in and made changes to the environment. Instead, this species lives in and around towns and avoids quiet, people-free areas. One of their characteristics is that they can burrow themselves into the soil with their hind limbs quite efficiently. Chanda (2002) reported that although this species has been found in north east India, no data are available on its natural history (Fig. 2.3).

(iv.) *Microhyla berdmorei* (Blyth, 1856): The distribution of *Microhyla berdmorei* ranges from Bangladesh to Indonesia. It inhabits various types of moist evergreen forest generally associated with hilly regions; also occurs in secondary growth. Breeding mainly takes place in standing waters. Chanda (2002) reported that they are very rare species and no data are available on their natural history. Therefore, more extensive study is necessary to know the actual status of the species (Fig.2.4).

In the present study, extensive survey on the distribution of *Euphlyctis cyanophlyctis*, *Hylarana nicobarensis*, *Kaloula pulchra* and *Microhyla berdmorei* was conducted throughout the years in 2005, 2006 and 2007 in all the eight districts of the state of Mizoram.

Sampling strategy: Extensive survey was made during the day and during the nocturnal censuses of oviposition sites and the habitat associations of tadpole species, by Visual Encounter Surveys (VES) with the help of head lamps, torch light, bamboo torch 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 5% 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 incorporated the following:

Data Sheet

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:	

Morphometric Measurements: Measurements of the frogs were carried out using a dial vernier caliper (Mitutoyo series No. 505-671) accurate to 0.02 mm. While majority of the frogs were released back, some were killed by over anesthetization with chloroform and then fixed in 5% formaldehyde for preservation, but before keeping in formaldehyde, a small incision was made on the lateral side of the abdomen for proper preservation. 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, Zoological Survey of India, Shillong and Department of Zoology, Mizoram University, Aizawl. 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:** Snout - vent length
- 2. SL:** Snout length (distance from the front of the eye to the tip of the snout)
- 3. EN:** Eye to nostril (distance from the front of the eye to the nostril)
- 4. SN:** Snout to nostril (distance from the nostril to the tip of snout)
- 5. TYE:** Distance from tympanum to the back of eye
- 6: INS:** Inter nasal space
- 7. IOD:** Inter orbital distance at narrowest point
- 8. UE:** Greatest transverse width of eyelid
- 9. ED:** Diameter of the exposed portion of the eyeball
- 10. HTYD:** Horizontal tympanum diameter
- 11. VTYD:** Vertical tympanum diameter
- 12. HL:** Head length (from the back of the mandible to the tip of snout).
- 13. HWN:** Head width at nostril
- 14. HWAE:** Head width at the anterior of the eye
- 15. HWPE:** Head width at the posterior of the eye
- 16. HWAJ:** Head width at angle of jaw
- 17. HDN:** Head depth at nostril
- 18. HDE:** Head depth at eye
- 19. HDAJ:** Head depth at angle of jaw
- 20. MN:** Distance from the back of mandible to the nostril
- 21. MAE:** Distance from the back of mandible to the anterior of the eye
- 22. MPE:** Distance from the back of mandible to the posterior of the eye

- 23. IAE:** Distance between the anterior of the eye
- 24. IPE:** Distance between the posterior of the eye
- 25. AG:** Axilla to groin distance
- 26. FLL:** Fore limb length (from proximal end of arm with to tip of longest finger)
- 27. HAL:** Hand length (from the base of outer palmar tubercle to tip of finger)
- 28. F₁:** Length of first finger (From the base of palm to tip of first finger)
- 29. F₂:** Length of second finger (From the base of palm to tip of second finger)
- 30. F₃:** Length of third finger (From the base of palm to tip of third finger)
- 31. F₄:** Length of fourth finger (From the base of palm to tip of fourth finger)
- 32. F_{1D}:** Diameter of the tip of first finger
- 33. F_{3D}:** Diameter of the tip of third finger
- 34. HLL:** From mid-ventral line of attachment of leg with body to tip of longest toe
- 35. TBL:** Tibia length
- 36. TBW:** Tibia width
- 37. TSL:** Tarsus length
- 38. FTL:** Foot length (proximal edge of inner metatarsal tubercle to tip of 4th toe)
- 39. T₁:** From base of foot to tip of longest toe
- 40. T₂:** From base of foot to tip of second toe
- 41. T₃:** From base of foot to tip of third toe
- 42. T₄:** From base of foot to tip of fourth toe
- 43. T₅:** From base of foot to tip of fifth toe
- 44. T_{1D}:** Diameter of the tip of first toe
- 45. T_{4D}:** Diameter of the tip of fourth toe

II. Breeding behavior, habit and habitat and ecological factors (temperatures of air and water, rainfall, relative humidity, pH and vegetations of the habitat) of the study sites:

To study the breeding behavior (courtship, mating calls, spawning and clutch size), habit and habitat, and the ecological factors of the breeding sites of *Euphlyctis cyanophlyctis*, *Hylarana nicobarensis*, *Kaloula pulchra* and *Microhyla berdmorei*, five study sites designated as study sites I, II, III, IV and V were selected from different places of Mizoram. To understand their breeding behaviour, the study sites were monitored during the years 2005, 2006 and 2007. The characteristic features of the study sites were as follows:

Study site I: It consists of three permanent ponds, located at Kawnpui (N 23° 58' 15.5"; E 92° 41' 30.9"; elevation = 310 m asl), Kolasib district (Fig. 3.2 & Fig. 3.3a-c). Study site I is used for monitoring *Euphlyctis cyanophlyctis*.

Study site II: It is a section of Tuitun stream (23° 58' 21.27" – 40.19" N and 92° 41' 05.51" – 10.35" E; elevations = 300 m – 325 m asl.), Kolasib district (Fig. 3.4 & Fig. 3.5a&b). *Hylarana nicobarensis* and *Microhyla berdmorei* were monitored in this site-II.

Study site III: This includes rock-pools on the stream-bed at Sihhmui (23° 47.913'N – 48.593' N and 92° 38.937' E – 39.203' E; elevation= 180 m – 184 m asl), Aizawl district(Fig. 3.6 & Fig. 3.10a&b). Study site-III is used for monitoring *Kaloula pulchra*.

Study site IV: It lies in the 23° 48' 24.66" N - 55.04" N and 92° 38' 44.51" – 39' 08.97" E along the stretch of Tlawng river, Aizawl district. The altitudes range from 35 m to 50 m asl (Fig.3.8 & Fig.3.9). Study site-IV is used for monitoring the

breeding behaviour and development of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis* and *Microhyla berdmorei*.

Study site V: This study site consists of temporary pools and rock-pools, located in the 23° 44.144' N and 92° 40.282' E at the altitude of 865m asl inside the campus of Mizoram University, Tanhril, Aizawl (Fig. 3.10 & Fig. 3.11a&b). It is also used for monitoring *Kaloula pulchra*.

To study the breeding behavior of the mentioned species, field survey was conducted in the evenings from 2:00 PM and continued till late at night depending on their breeding season. Audio Encounter Surveys (AES) are used to identify locations where adult animals are attempting to breed. During the above survey period each selected sampling site was covered at different times of the day (from 2:00 PM to 11:00 AM on the following day) in order to record their breeding behaviours. Acoustic Encounter Surveys (AES) are used to identify exact locations where adult animals are attempting to breed. Egg mass and larval surveys provide evidence that mating occurred. The number of egg masses is also an indication of the number of adults that bred at that location. Torch light, headlamp and bamboo torch were used to locate and count the number of males and females of the frogs at their breeding sites. Field sampling was carried out at fortnightly intervals; each sampling session spanning over two to three continuous days.

Breeding behavior, amplexing pairs and freshly spawned eggs were studied and documented with the help of photographic and video cameras. The amplexing pairs and their newly laid were photographed with a camera, Sony Cyber-shot DSC-H10 (Super Steady Shot). Rainfall data for these five study sites were obtained from the Directorate of Agriculture & Minor Irrigation, Aizawl. The duration when the

breeding pairs remained in amplexus was noted down and the temperatures of atmospheric as well as water and relative humidity of the study sites were recorded. Water pH was measured with the help of pH pen and digital pH meter.

The numbers of egg clutches at the breeding sites were also counted. Egg clutches laid by the amplexing pairs were collected from the field and their measurements were taken and the clutch size was determined. The total numbers of eggs in the obtained clutches were counted and the morphometric measurements of amplexing pairs were measured. The data were analysed statistically with the help of statistical software tools SPSS (7.5.1 version) and OriginPro 8 SRO (8.0724 version).

Mating calls were recorded with the help of digital voice recorder Samsung SVR 380 (FM frequency range 87.5-108 MHz). The sampling rate used to convert the signals to digital format was 8 KHz with 16-bit precision. The oscillogram was prepared and analysed 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 cycle of these four species was studied with special reference to the breeding behaviour in relation to some environmental factors like temperature, rainfall and humidity. To study the histology of testes and ovaries during the breeding season, adult frogs were collected from the field and brought to the

laboratory. On reaching the laboratory ovaries from the females and testes from the males were excised and immediately fixed in Bouin's solution (24 hours). Fixation was done immediately so as to avoid post-mortem changes. The gonads were then dehydrated through alcoholic grades (30%, 50%, 70%, 90% and 100%), cleared in xylene and embedded in paraffin wax, serially sectioned at 7-8 μ thickness with the help of Microtome, and stained with haematoxylin and counter-stained with eosin. After mounted with DPX, 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.

III. Development and metamorphosis:

To study the development and metamorphosis of the four species, the investigation was conducted in the natural environment at the study sites II and IV for studying *Hylarana nicobariensis*; I and IV for *Euphlyctis cyanophlyctis*; study sites II and IV for *Microhyla berdmorei* and III and V for *Kaloula pulchra*. These five study sites were found to be excellent breeding grounds and served as good habitat for the development and metamorphosis of these four species. Amplexing pairs from the above study sites were collected and brought to the laboratory and allowed to lay their eggs in the laboratory and were maintained in a plastic tray containing pond water to allow further development and metamorphosis. During the years 2005, 2006 and 2007 the freshly spawned eggs were also collected during the breeding phase immediately after they were laid at the study sites and brought to the laboratory for further studies. Development of the eggs was also observed with the help of a stereoscopic dissecting binocular microscope during field observations and

in the laboratory condition. Some egg masses were fixed immediately in the field in 5 % formaldehyde, and on reaching the laboratory the eggs were separated from the masses and counted to know the clutch size.

Temperature and pH of water was maintained as in the natural condition and the pond water was changed every alternate day. The rate of development was observed under a stereoscopic dissecting binocular microscope (Labomed CSM2). The time of onset of each new stage was noted and some developmental stages were fixed in a mixture of 70% alcohol and 4% formaldehyde in the ratio of 1:1. Staging of the anuran embryos and larvae of both the species was carried out on the basis of a new external morphological change as per the criteria described by Gosner (1960). Photographs of the developmental stages were taken with the help of microscope (Labomed CSM2) with photographic attachments.

The hatched tadpoles were reared in a plastic tray containing pond water collected from the study sites and fed daily with algae gathered from the breeding sites and boiled cabbage. The temperature in the laboratory conditions was monitored. In order to know the stages attained by the developing tadpoles, regular observation was conducted depending on the rate of development. Simultaneous observation was conducted both in the natural and laboratory conditions and the five study sites were visited at a regular interval of every three days to observe and record the development and metamorphosis of the four species in the natural environment by marking the location and net the egg masses, and making an enclosure. The size of the enclosure for *Kaloula pulchra* was 120 cm (Fig. 3.12c) in circumference and for *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis* and *Microhyla berdmorei*, it varies from 50 cm X 50 cm to 100 cm X 200 cm (Fig. 3.12a, b and d, respectively).

Throughout the study period from the month of January 2005 to December 2007, samples of water from each study site were collected in plastic bottle at fortnightly interval for the analysis of water temperature and pH. Water temperature was recorded in the field and pH was recorded with the help of pH pen (S252873 HANNA Instrument) and digital pH meter in the field and laboratory respectively. Simultaneously, the ecological factors like air temperatures and relative humidity of the study sites were recorded. Rainfall data for these five study sites were obtained from the Directorate of Agriculture & Minor Irrigation, Aizawl.

IV. Food and feeding behavior in relation to oral structures and intestines:

The tadpoles were collected from the study sites and were staged according to Gosner (1960). After collection in the field, the tadpoles were immediately fixed in 4% formaldehyde and autopsied for analysis of gut content. The foregut of a tadpole close to the oesophagus was cut and its contents teased on to a glass slide or petridish. Gut wall and visible portions of lining were removed with several drops of distilled water. The gut contents were spread as thinly as possible; a cover slip placed over it and the edges sealed. Each smear was observed within a few days of preparation using a compound microscope. The remainder that could not be identified will be placed in a vial containing 4% buffered formalin. Identification on the food items of the tadpoles was made following the method of Turner (1892), Edmonson (1959), Needham and Needham (1972), and Fritsch (1979). The feeding habits of adults will be studied by removing the stomach contents and analysed under a stereoscopic microscope. Histology of the developing gut walls of tadpole and adult will be studied by using the method given by Rugh (1962).

The oral structure of the selected developing tadpoles were studied using light microscopy, stereo-scopic binocular microscope following the criteria of Altig and McDiarmid (1999), and some also with Scanning Electron Microscopy.

For scanning electron microscopy, the samples were washed with double distilled water, fixed in 2.5% Glutaraldehyde solution (Prepared in 0.1M Na-Cacodylate buffer) for 4 hours and post-fixed in 1% Osmium tetroxide buffered (0.1M Na-Cacodylate buffer) for 1 hour at 4°C. The pH of the 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-35 CF (Jeol) scanning electron microscope at an accelerating voltage of 20KV. Working distance (WD) was at 15mm and the necessary tilting angles were used to view the frontal portion of the oral structures.

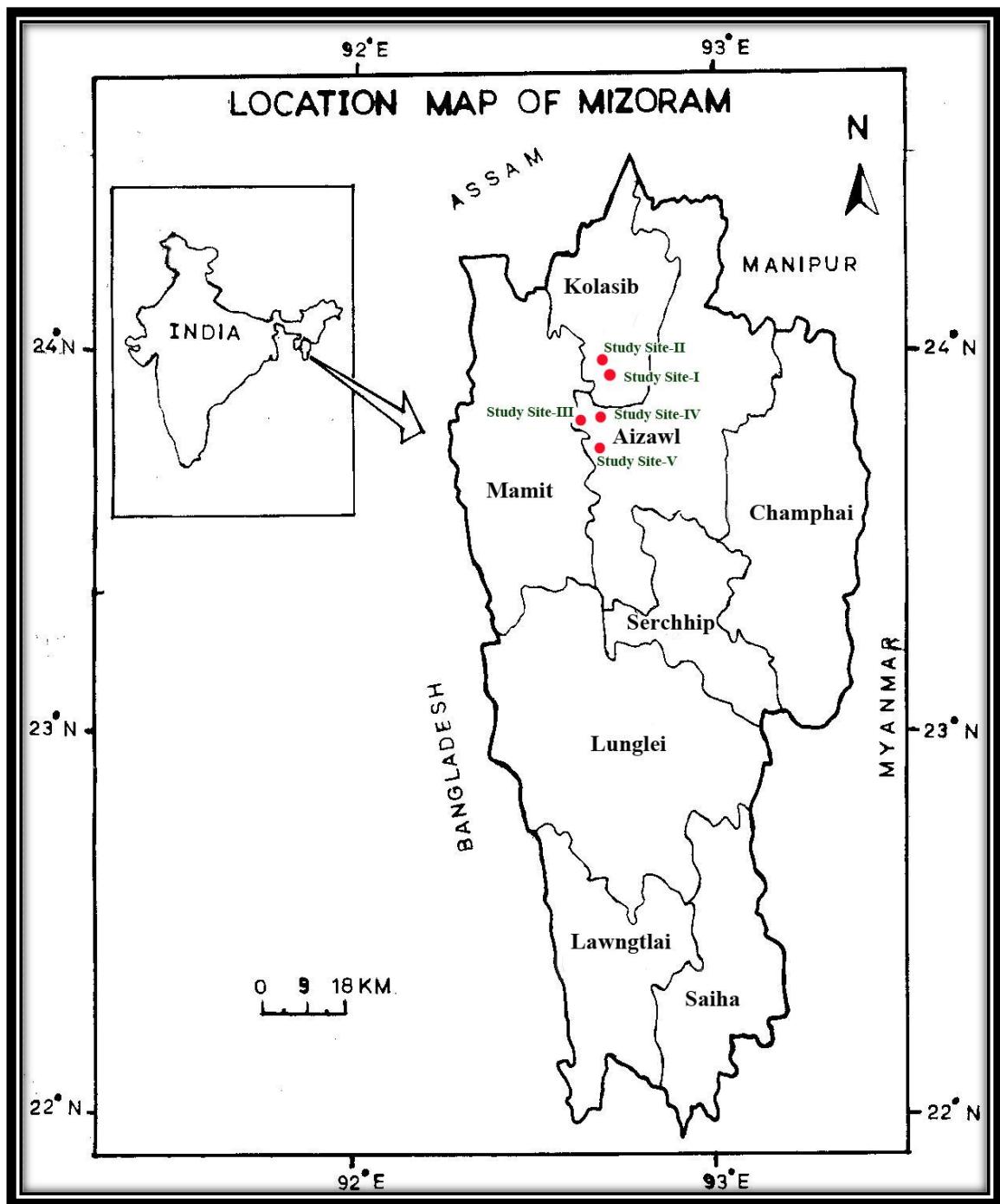


Fig.3.1. Map of Mizoram showing all the eight districts and location of the study sites.

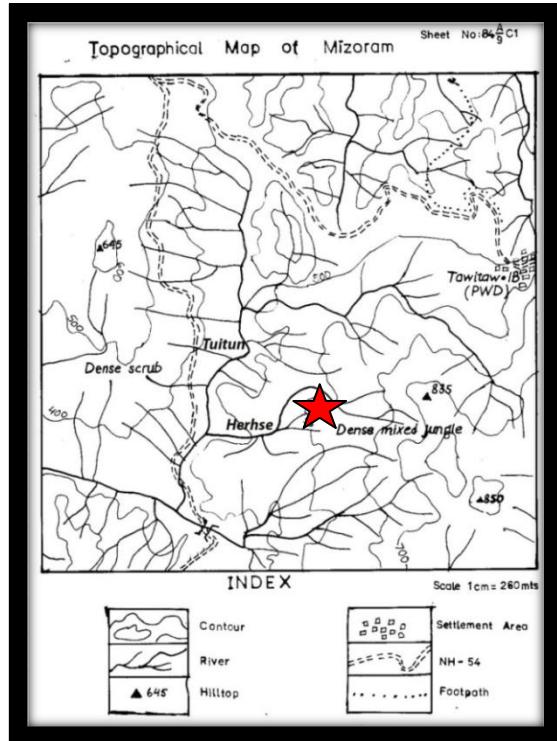


Fig. 3.2: Location of study site - I at Kawnpu ($N\ 23^{\circ}\ 58'\ 15.5''$; $E\ 92^{\circ}\ 41'\ 30.9''$; elevation = 310 m asl), Kolasib district.



Fig. 3.3 (a-c): Study site - I consists of three permanent ponds, named as pond-I, pond-II and pond-III used for monitoring breeding and development of *Euphlyctis cyanophlyctis*.

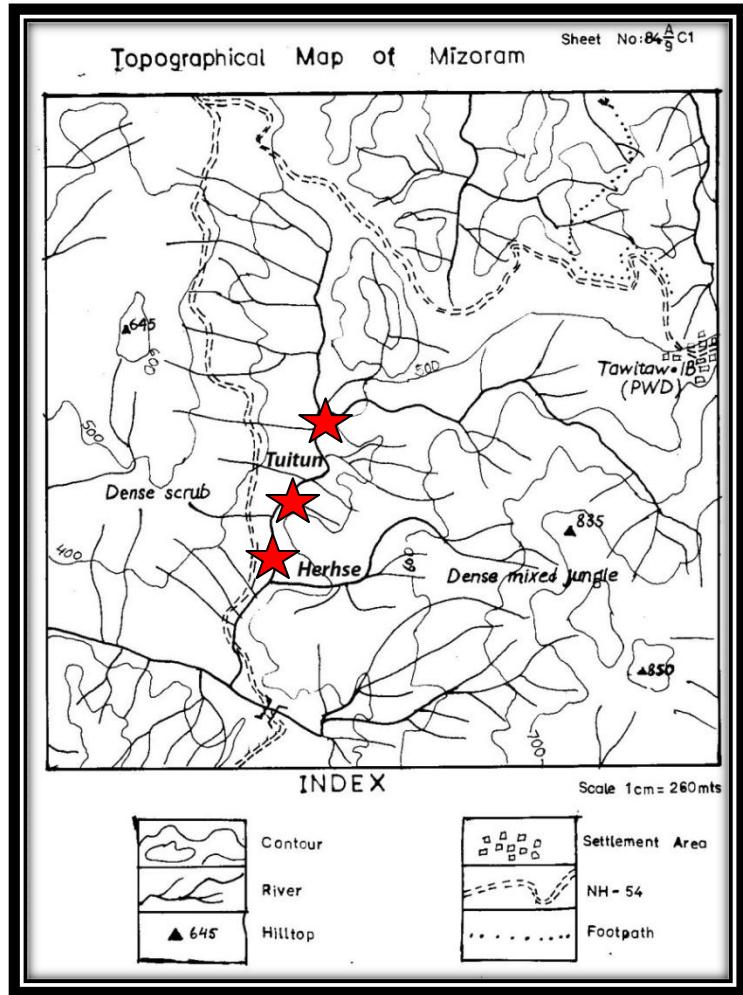


Fig. 3.4: Location of study site - II at Tuitun stream ($23^{\circ} 58'21.27''$ – $40.19''$ N; $92^{\circ} 41' 05.51''$ – $10.35''$ E; elevations = 300 m – 325 m asl.), Kolasib district.



Fig. 3.5 (a & b): Microhabitats at study site - II (Tuitun stream) used for monitoring breeding and development of *Hylarana nicobariensis* and *Microhyla berdmorei*.

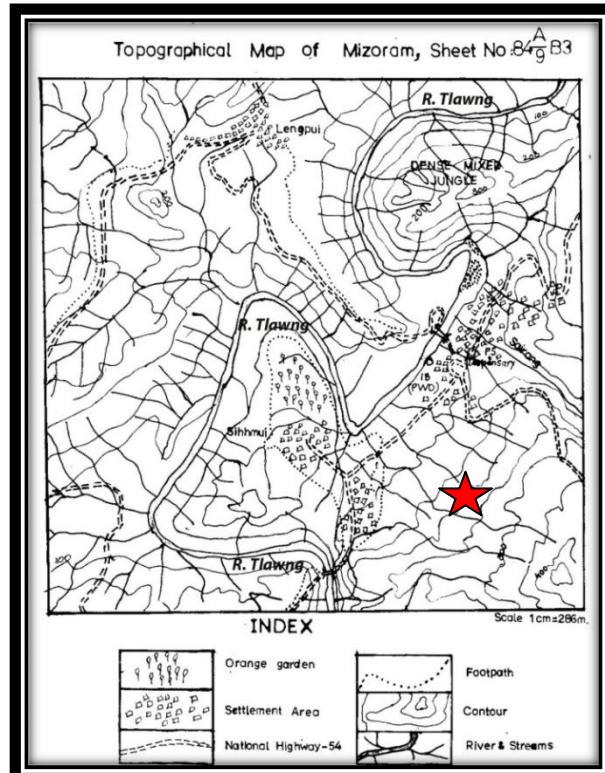


Fig.3.6: Location of study site III at Sihhmui ($23^{\circ}47.913'N$ – $92^{\circ}38.937'E$ – $39.203'E$; 180 m – 184 m asl), Aizawl district.



Fig.3.7 (a & b): Rock-pools at study site III used for monitoring breeding and development of *Kaloula pulchra*.

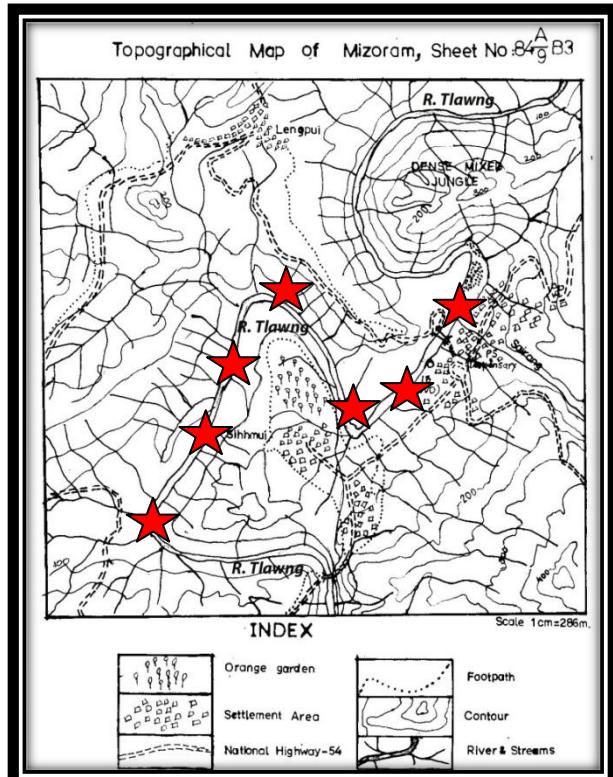


Fig. 3.8: Location of study site IV along the stretch of Tlawng river ($23^{\circ} 48' 24.66''$ N - $55.04''$ N; $92^{\circ} 38' 44.51''$ – $39' 08.97''$ E; 35 m – 50 m asl.), Aizawl district.



Fig. 3.9: Study site IV at Tlawng river used for monitoring breeding and development of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis* and *Microhyla berdmorei*.

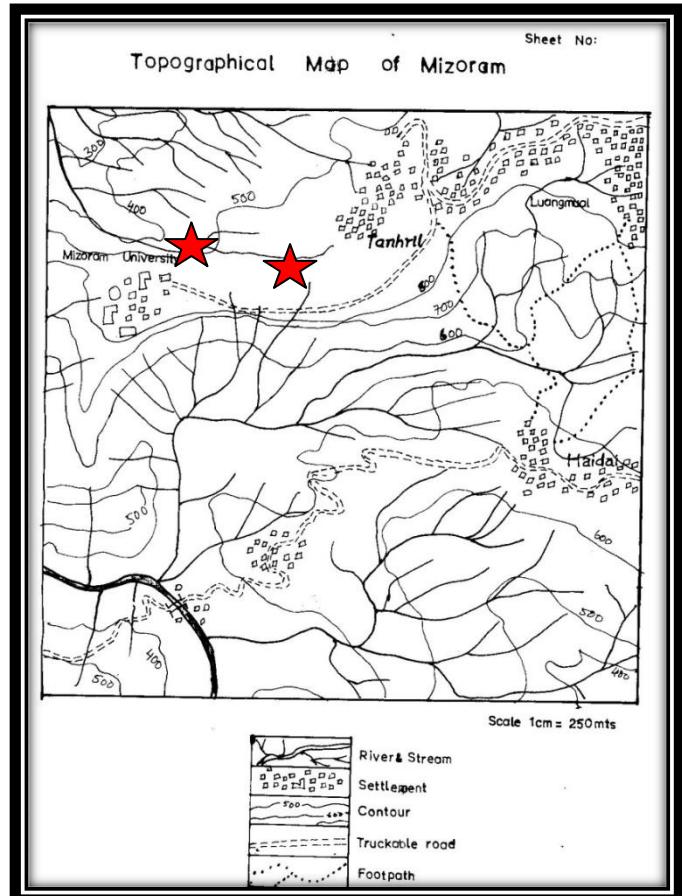


Fig. 3.10: Location of study site V inside the campus of MZU ($23^{\circ} 44.144'N$; $92^{\circ} 40.282'E$; 865m asl) at Tanhril, Aizawl.



Fig. 3.11 (a & b): Rock-pools and temporary pool at the study site V used for monitoring breeding and development of *Kaloula pulchra*.

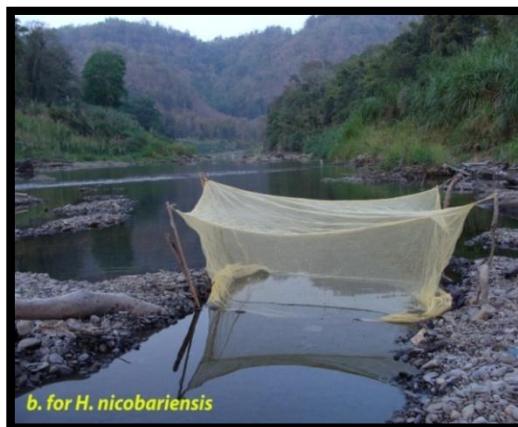


Fig. 3.12 (a-d): Enclosures for monitoring larval development of the four species in their natural microhabitats.

CHAPTER 4

RESULTS

CHAPTER 4

RESULTS

I. Distribution of Ranid and Microhylid anurans prevalent in Mizoram

Mizoram has an abundant growth of vegetation. Its tropical location, which furnishes conductive climatic conditions, such as an adequate rainfall, moderate temperature, etc. favours the luxuriant growth of vegetation which is important for the habitat and distribution of amphibians. The type of vegetation which thrives in Mizoram ranges from tropical to sub-tropical. There is a marked difference between vegetation of the western and eastern part of the state. The differences in natural vegetation are due to variation in topography, soil and climatic conditions.

During the study period i.e. 2004 to 2007, different parts of Mizoram were surveyed. All the eight districts i.e. Aizawl District, Champhai District, Kolasib District, Lawngtlai District, Lunglei District, Mamit District, Saiha District and Serchhip District are visited for species collection and documentation. In the present investigation, *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* found to be prevalent in Mizoram were collected/encountered from different areas (Fig. 4.1). The study conducted contributed more information on the distribution of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* from thirty (30) localities in various parts of Mizoram, which include the following:

Aizawl, Rung dil, Sairang, Sihmu, Tam dil, Durlui, Tuirial, Tuirini, Tuivawl, Champhai, Tuichang, Tuipui, Khawzawl, Kawlkulh, Khawhai, Bilkhawthlir, Buhchang, Tuitun, Kawnpu, Lawngtlai, Theiriat, Ramlaitui, Lengpui,

Tut, Zawlnuam, Khankawn, New Latawh, Chhingchhip, Mat and Thenzawl. The distributions of selected species, *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* in Mizoram as documented from the present survey are shown in the Table 1 and Fig.4.2. The elevation of the survey sites were categorized as follows: high elevation regions ranging between 1000 m – 1500 m, mid-elevation between 500 m – 1000 m and low elevation between 40 m – 500 m above sea level (asl). A brief description of the survey areas and sites of collections from different districts of Mizoram are as follows:

(1.) Aizawl District:

Aizawl: The capital of Mizoram state is located ($23^{\circ}43'$ - $44'$ N and $92^{\circ} 40'$ - $44'$ E) at an altitude of 743-965 m asl. *Kaloula pulchra* (1 male) and *Euphlyctis cyanophlyctis* (7 males and 5 females) were collected from a permanent pond inside the picnic spot of Local Administrative Department and also from Lawibual, Mualpuui locality. *Hylarana nicobariensis* (1 male), *Kaloula pulchra* (2 males and 1 female) and *Microhyla berdmorei* (3 males and 1 female) were encountered and collected from the streams, temporary pools and water tanks in and around Mizoram University campus, at Tanhril locality.

Rung dil: It consists of two lakes located about 10 m apart and 127 km from Aizawl. These two lakes lies within ($23^{\circ}59'$ N and $93^{\circ}00'$ E) at an elevation of about 332 m asl. *Euphlyctis cyanophlyctis* (2 males and 1 female), *Hylarana nicobariensis* (3 males and 1 female) and *Kaloula pulchra* (1 male) were encountered and collected from the surrounding virgin forest, streams and paddy fields.

Sairang: It is located ($23^{\circ}48'$ N and $92^{\circ}37'$ - $39'$ E) at an altitude of 50 m – 80 m asl. It is about 25 km from Aizawl and is famous for the river, Tlawng (the longest river

in Mizoram) which runs through it from south to north. *Euphlyctis cyanophlyctis* (3 males and 2 females), *Hylarana nicobariensis* (8 males and 3 females), *Kaloula pulchra* (2 males and 1 female) and *Microhyla berdmorei* (6 males and 3 females) were collected and quite common among the floors of teak (*Tectona grandis*) plantation and *Melocana bambusoides*, inside and along the river and its tributaries in this area.

Sihmuui: Located (23°47'N and 92°39'E) at an altitude of 180 m asl is about 27 km from Aizawl. *Euphlyctis cyanophlyctis* (4 males and 2 females), *Hylarana nicobariensis* (5 males and 3 females), *Kaloula pulchra* (5 males and 4 females) and *Microhyla berdmorei* (4 males) were encountered and collected from secondary forest, plantation and along the streams found in this area.

Tam dil (lake): It is the second largest lake in Mizoram. It is famous not only for tourist spot, but also for being one of the two National Wetlands in Mizoram. It lies in the 23°44'N and 92°57'E (745 m asl), about 80 km from Aizawl. *Euphlyctis cyanophlyctis* (7 males and 2 females) and *Hylarana nicobariensis* (6 males and 4 females) were encountered and collected from the lake and its surrounding fish nursery ponds, ditches, streams and forest area.

Durlui stream: It is one of the tributaries of Tlawng river, running from east to westward located at 23°53'N and 92°39'E at an elevation of 103 m asl, about 45 km from Aizawl. This stream flows through hilly terrain and tropical semi-evergreen forest. They carry clear water without turbidity. The stream bed is rocky, shallow or temporarily deep in some areas. The stream may comprises submerged vegetation, rocks and huge boulders, steep waterfalls, rapids and pools. *Euphlyctis*

cyanophlyctis (3 males), *Hylarana nicobariensis* (3 males and 2 females) and *Microhyla berdmorei* (2 males) were encountered and collected from this stream.

Tuivawl river: It is a river that runs from south to north and the sites of collection were located ($23^{\circ}36'N$ and $93^{\circ}01'E$) at an altitude of 488 m asl which is about 97 km from Aizawl. *Euphlyctis cyanophlyctis* (3 males and 1 female), *Hylarana nicobariensis* (5 males and 2 females) and *Microhyla berdmorei* (2 males) were encountered and collected from this slow-flowing river.

Tuirial river: The surveyed sites in this river is located ($23^{\circ}43'N$ and $92^{\circ}47'E$) 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. *Euphlyctis cyanophlyctis* (4 males), *Hylarana nicobariensis* (3 males and 1 female) and *Microhyla berdmorei* (2 males) were encountered and collected along this river.

Tuirini river: The collection sites in this river lie within $23^{\circ}41'N$ and $92^{\circ}53'E$ 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. *Euphlyctis cyanophlyctis* (2 males and 2 females), *Hylarana nicobarensis* (1 male and 3 females) and *Microhyla berdmorei* (1 male) were collected from this river.

(2.) Champhai District:

Champhai: It is the Capital of Champhai district, located at a distance of 194 km from Aizawl. The sampling sites in this area located between $23^{\circ}31'$ - $32'N$ and $93^{\circ}15'$ - $16'E$, with the altitude ranging from 951 m to 1460 m asl. *Euphlyctis cyanophlyctis* (3 males and 2 females), *Hylarana nicobariensis* (4 males and 1

female) and *Kaloula pulchra* (1 male) were collected from the paddy field and their adjacent streams and surrounding secondary forest.

Tuichang: It is about 140 km from Aizawl and the surveyed sites in this river were within 23°32'N and 93°06'E, at an altitude of 603 in the Champhai district. It originates from the northern side of Mizoram and continues towards the south to join Tuipui river. *Euphlyctis cyanophlyctis* (3 males and 2 females), *Hylarana nicobariensis* (2 males and 1 female) and *Microhyla berdmorei* (2 males) were documented and collected from this river.

Tuipui: This river flows toward south and later confluents with Tiau river which is the boundary between Mizoram and Myanmar. The collection sites in this river were located (23°27'N and 93°10'E) at an altitude of 708 m asl in the Champhai district. It is about 172 km from Aizawl. *Euphlyctis cyanophlyctis* (2 males) and *Hylarana nicobariensis* (1 male and 1 female) were encountered from this river.

Khawzawl: It is the second largest town in the Champhai district, located (23°32'N and 93°10'E) at the elevation between 1158 m – 1257 m asl. It is about 152 km from Aizawl. *Euphlyctis cyanophlyctis* (4 males and 1 female) and *Kaloula pulchra* (2 males) were collected.

Kawlkulh: It is located (23°43'N and 93°05'E) at an altitude of 965 m asl in the Champhai district. It is about 130 km from Aizawl. *Euphlyctis cyanophlyctis* (3 males) were collected from a pond called as Lun dil.

Khawhai: It is located (23°22'N and 93°07'E) at an altitude of 1378 m asl in the Champhai district. It is about 184 km from Aizawl. *Euphlyctis cyanophlyctis* (4 males and 2 female) were collected from a lake known as Khawhai dil.

(3.) Kolasib District:

Bilkhawthlir: It is a village located between 24°20'N and 92°41'E at an altitude of 65 m asl in the Kolasib district. It is about 121 km from Aizawl. It is surrounded by paddy fields and stream-fed fish ponds from which *Euphlyctis cyanophlyctis* (2 males), *Hylarana nicobariensis* (1 male), *Kaloula pulchra* (1 male) and *Microhyla berdmorei* (3 males and 2 females) were encountered and collected.

Tuitun: It is a stream, located in 23°58'N and 92°41'E at an elevation of 308 m – 326 m asl at Kawnpui area, Kolasib district, about 60 km from Aizawl. *Euphlyctis cyanophlyctis* (4 males and 2 females), *Hylarana nicobariensis* (8 males and 7 females), *Kaloula pulchra* (1 male) and *Microhyla berdmorei* (9 males and 8 females) were encountered and collected from this stream.

Kawnpui: Kawnpui town is located (23°56'N and 92°41'E) at an altitude of 910 m asl, Kolasib district. It is about 69 km from Aizawl. *Euphlyctis cyanophlyctis* (2 males), *Hylarana nicobariensis* (3 males and 1 female) and *Kaloula pulchra* (4 males and 3 females) were collected from cemented tanks and streams flowing within the town.

Buhchang: It is a village flanks by paddy fields and fish ponds, located (24°20'N and 92°39'E) at an elevation of 46 m asl in the Kolasib district. *Euphlyctis cyanophlyctis* (2 males), *Hylarana nicobariensis* (1 male), *Kaloula pulchra* (1 female) and *Microhyla berdmorei* (1 male) were collected around this area.

(4.) Lawngtlai District:

Lawngtlai: It is a 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. *Euphlyctis cyanophlyctis* (2 males), *Hylarana nicobariensis* (2 males and 1 female), *Kaloula*

pulchra (1 male) and *Microhyla berdmorei* (1 male and 1 female) were collected from the surrounding streams and forest.

(5.) Lunglei District:

Theiriat: Being located in the Lunglei district ($22^{\circ}55' - 56'N$ and $92^{\circ}45'E$) at the elevation between 1048 m – 1060 m asl. It is about 225 km from Aizawl. *Euphlyctis cyanophlyctis* (3 males), *Hylarana nicobariensis* (2 males) and *Microhyla berdmorei* (2 males) were collected from the surrounding streams and forested area. Tlawng river originates near this locality.

Ramlaitui: It is also located inside the Lunglei district. It is about 150 km from Aizawl. *Euphlyctis cyanophlyctis* (1 male), *Kaloula pulchra* (1 male) and *Microhyla berdmorei* (2 males and 1 female) were collected from the ponds and streams in this village area ($23^{\circ}20'N$ and $92^{\circ}46'E$) at an altitude of 750 m asl.

(6.) Mamit District:

Tut river: This river originates from Lunglei district and flows south to north direction. The sampling sites in Tut located ($23^{\circ}46'N$ and $92^{\circ}31'E$) at an altitude of 74 m asl, in the Mamit district, about 76 km from Aizawl. A number of streams and thick forest covers are present which gives an ideal habitat for anurans. *Euphlyctis cyanophlyctis* (3 males), *Hylarana nicobariensis* (1 male and 2 females) and *Microhyla berdmorei* (2 males) were collected from this river and its surrounding tropical wet-evergreen forest and its tributaries.

Lengpui: It is located ($23^{\circ}49' - 50'N$ and $92^{\circ}37'E$) at the elevation of 390 m – 400 m asl, at Mamit district. It is famous due to the presence of the one and only Airport in Mizoram. The surveyed spots within and around this village consists of fish ponds, streams and their surrounding vegetations. *Euphlyctis cyanophlyctis* (4 males and 2

females), *Hylarana nicobariensis* (3 males and 1 female), *Kaloula pulchra* (1 male) and *Microhyla berdmorei* (6 males and 3 females) were encountered and collected from this area.

Zawlnuam: It is located on the northwestern part of the state bordering with Tripura state about 173 km from Aizawl and located ($24^{\circ}15'N$ and $92^{\circ}30'E$) at an altitude of 180 m asl in the Mamit district. Dense forest of tropical wet-evergreen are still persists around this small town. *Euphlyctis cyanophlyctis* (2 males), *Kaloula pulchra* (1 male and 1 female) and *Microhyla berdmorei* (2 males and 1 female) were collected from this area.

(7.) Saiha District:

Khankawn: It is located ($22^{\circ}22'N$ and $92^{\circ}57'E$) at an altitude of 193 m asl in the Saiha district. It is about 370 km from Aizawl. *Euphlyctis cyanophlyctis* (4 males and 1 female), *Hylarana nicobariensis* (5 males and 3 females) and *Microhyla berdmorei* (3 males and 2 females) were collected from the streams in this locality.

New Latawh: It is about 340 km from Aizawl. It is located ($22^{\circ}22'N$ and $92^{\circ}55'E$) near Chhimtuipui river, at an altitude of 458 m asl in the Saiha district. It is a small village with thick forest covers and a few streams running near the edges of these forests. *Euphlyctis cyanophlyctis* (1 male) and *Hylarana nicobariensis* (3 males and 2 females) were collected from these streams near the village.

(8.) Serchhip District:

Chhingchhip: It is located ($23^{\circ}27'N$ and $92^{\circ}51'E$) at the elevation of 1113 m asl in the Serchhip district. It is about 82 km from Aizawl. *Euphlyctis cyanophlyctis* (5 males and 4 females), *Hylarana nicobariensis* (3 males and 1 female) and *Microhyla berdmorei* (3 males) were collected from the sites along the streams.

Mat: It is a river that flows north to south originates from the central part of Aizawl district, crossing Serchhip district and later confluents with Chhimtuipui river at the district boundary between Lunglei district and Saiha district. The collection sites in this river were located between $23^{\circ}27'N$ and $92^{\circ}50'E$ at an altitude of 651 m, in the Serchhip district, which is about 90 km from Aizawl. *Euphlyctis cyanophlyctis* (3 males), *Hylarana nicobariensis* (2 males), *Kaloula pulchra* (1 male) and *Microhyla berdmorei* (2 males and 3 females) were collected from this river basin and its surrounding paddy rice fields.

Thenzawl: It is a town located ($23^{\circ}17'N$ and $92^{\circ}46'E$) at the elevation between 741 m – 810 m asl, in the Serchhip district, about 120 km from Aizawl. The terrain of Thenzawl area is plain and most of the area is utilized for plantation and fishery. *Euphlyctis cyanophlyctis* (4 males and 3 females), *Hylarana nicobariensis* (2 males), *Kaloula pulchra* (2 males) and *Microhyla berdmorei* (2 males and 1 female) were collected from this area.

From the result, it was found that all the four species were collected from 7 districts, Aizawl, Champhai, Kolasib, Lawngtlai, Lunglei, Mamit and Serchhip. In case of Saiha district, other three species were reported except *Kaloula pulchra*. The present investigation provides more information about the distribution of these four species in different areas of Mizoram. During the present survey, *Euphlyctis cyanophlyctis* was collected from all the thirty surveyed sites throughout all the eight districts (Table 1 & Fig. 4.2), from low altitude to high altitude (46 m – 1460 m asl.) between latitude $22^{\circ}22'N$ - $24^{\circ}20'N$ and longitude $92^{\circ}30'E$ - $93^{\circ}10'E$, and from different habitats, marshes, stream, river edges, pools, ponds, paddy fields, etc. throughout the year.

Hylarana nicobariensis was collected from the twenty six surveyed sites, Aizawl, Rungdil, Sairang, Sihmu, Tam dil, Dur lui, Tuivawl, Tuirial, Tuirini, Lengpui, Champhai, Tuichang, Tuipui, Bilkawthlir, Buhchang, Kawnpui, Tuitun, Tut, Zawlnuam, Khankawn, New Latawh, Lawngtlai, Theiriat, Chhingchhip, Mat and Thenzawl (Table 1 & Fig. 4.2), with the elevation ranging from 46 m to 1460 m asl between latitude 22°22'N - 24°20'N and longitude 92°30'E - 93°10'E, and from different lotic habitats and their adjacent water bodies from September to April.

In the present survey, *Kaloula pulchra* was encountered naturally in a wide variety of habitats, from populated villages, to rice fields, to leaf-covered forest floors. During breeding season, they were collected mostly from rain-fed pools, water holes, permanent ponds, paddy fields and puddles surrounded by vegetation or forested area and cemented tanks in residential areas. It was found that although *Kaloula pulchra* lives near human habitation, they were rarely seen usually underground frog in the wild. However, in the present investigation, *Kaloula pulchra* was collected from sixteen surveyed sites of Mizoram (Aizawl, Rungdil, Sairang, Sihmu, Lengpui, Champhai, Khawzawl, Bilkawthlir, Kawnpui, Tuitun, Buhchang, Lawngtlai, Ramlaitui, Zawlnuam, Mat and Thenzawl) as shown in the Table 1 & Fig. 4.2. The above collection sites ranged from lower altitude to mid-altitude between 46 m – 910 m asl in the Aizawl district and Kolasib district, from lower altitude between 180 m – 400 m asl in Mamit district, mid- altitude regions ranging between 651 m – 847 m asl in Lawngtlai, Lunglei and Serchhip districts. Interestingly, they were collected from high altitude ranging between 1150 m to 1456 m asl in Champhai district, between latitude 22°31'N - 24°20'N and longitude 92°30'E - 93°10'E.

Microhyla berdmorei, a small narrow-mouthed frog was collected from rain-fed pools, ditches, permanent ponds, water holes, littoral region of the forest streams and rivers shaded and/or covered by surrounding vegetations and among bushes surrounding water bodies during its breeding period and also from the cemented tanks near residential areas. During the present study, it was collected from twenty one surveyed sites, i.e. Aizawl, Sairang, Sihhmui, Lengpui, Durlui, Tuivawl, Tuirial, Tuirini, Tuichang, Champhai, Bilkhawthlir, Tuitun, Buhchang, Lawngtlai, Ramlaitui, Theiriat, Tut, Zawlnuam, Khankawn, Mat and Thenzawl (Table 1 & Fig. 4.2). They were collected from lower altitude regions ranging from 46 m – 326 m asl in Kolasib and Saiha districts, from lower to mid altitude regions ranging from 47 m – 865 m asl in Aizawl and Mamit districts, from mid altitude ranging from 651 m – 847 m asl in Lawngtlai and Serchhip districts and also from mid to high altitude regions ranging from 603 m – 1460 m asl in Champhai and Lunglei districts, between 22°22'N - 24°20'N and longitude 92°30'E - 93°16'E.

Description and morphometric measurement of the specimens:

The present study deals with four selected anurans (ranids and microhylids) prevalent in Mizoram, i.e. *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* that breed in different season in Mizoram.

1. *Euphlyctis cyanophlyctis* (Schneider, 1799):

Diagnosis: Colour greyish to olive above, with dark olive round spots or marblings (Fig. 4.3a&b). A more or less prominent, dark, light-edged band present along each flank and on the anterior and posterior parts of thighs but may be absent

in some adults. Limbs are with incomplete dark bands. Ventral surface white or pale yellowish, marbled spotted, dotted or vermiculated with blackish. Most young specimens have the ventral surface almost white, without any trace of a few scattered fine dots. Dorsum is usually with small tubercles, warts and rows of pores. Ventral surface is almost smooth. There is a dorso-lateral row that extends from the eye to the groin and similar ventro-lateral rows that meet posteriorly. Curved rows that extend from the forelimb insertion to the pectoral region and rows that extend to the neck.

Description: Snout-vent length (SVL) of males ranges from 32.20 mm - 46.80 mm and females 31.23 mm - 61.15 mm (Table 2). Head slightly broader than long, depressed; snout pointed, slightly projecting beyond mouth; canthus rostralis indistinct; loreal region oblique and concave; nostrils equidistant from eyes and tip of snout; internarial space is more or less equal to interorbital width; tympanum distinct, more than half of eyes, separated from the latter by a space about $\frac{1}{4}$ of tympanic diameter; vomerine teeth small, oblique in position on a level with the posterior border of choanae, equidistant from each other and from choanae (Fig. 4.4). Forelimbs moderately long; fingers pointed, free with minute dermal border except in fourth finger; first and second fingers equal, third finger longest, longer than snout; subarticular tubercles small and feebly prominent (Fig. 4.5a). Hindlimbs stout and long; tibiotarsal articulation reaching between eyes and nostril/tympanum; heels not meeting when hindlimbs folded at right angles to body; tibia 2½ to 3 times as long as broad, less than half the length of snout to vent; toes fully webbed with webbing formula: I₀₋₀II₀₋₀III₀₋₀IV₀₋₀V (Fig. 4.5b); tips of toes swollen into small discs; outer metatarsals separated at the base; subarticular tubercles small, feebly

prominent; a small, prominent, pointed digitiform inner metatarsal tubercle present; outer metatarsal tubercle absent; a strong dermal fringe on the outer toe.

Sexual dimorphism: Males slightly smaller than females with a grey or blackish external vocal sac on each side, projecting through a slit close to the posterior half of the mandibular ramus, the slit as long as or a little longer than the eye. During the breeding season, males are usually with a weakly developed nuptial pad at the base of the first finger. The venter in female is usually marbled while it is white in male.

Tadpole Description:

Diagnosis: Body olive to grey-brown with loosely scattered black spots, tail muscle and fins mostly black with small, creamy patches, dorsal tail fin higher than ventral one, transparent marginal papillae.

Morphology: Body large, with broad bulky ellipsoidal belly, broadest at its posterior half; snout rounded, slightly depressed; eyes dorsolateral, nostrils dorsal, open, equidistant between snout tip than eye; spiracle sinistral, extended as a short tapered tube, inner wall free from body, spiracular opening directed posterio-dorsally; medial vent tube with lateral displacement towards dextral, continuous with ventral fin. Tail lanceolate, dorsal fin margin weakly convex, both margins tapering abruptly at distal quarter towards a narrowly pointed tip. Dorsal fin originating at body-tail junction, gently sloping towards mid-tail convex, dorsal fin slightly deeper than ventral. Naso-lacrymal groove present.

Colour/Markings: Dorsum and flanks olive to grey-brown with scattered black spots, tail muscle and fins mostly black with small, creamy patches; venters yellowish white with silvery sides of body.

Oral Disc: Mouth ventral, sub-terminal, marginal papillae of anterior labium confined to lateral corners, consisting of three to four simple, triangular fleshy extensions on each side; marginal papillae of posterior labium continuous, consisting of transparent, elongated, tongue like extensions; short, bulbous inframarginal papillae present; both jaw sheaths serrated at edge, keratinised at margins. Labial teeth row formula (LTRF): 1/2.

Habit and Habitat: *Euphlyctis cyanophlyctis* are found to be active both day and night. They were seen basking in the sun by sitting on the bank of water or aquatic plant or by floating on the water surface. During the present survey, they were encountered among the littoral edges of ponds, lakes, streams and rivers and also commonly found in other stagnant water bodies like ditches on the roadside, paddy fields, water canals and tanks.

Distribution in Mizoram: In the present investigation, *Euphlyctis cyanophlyctis* was collected from all the 30 surveyed sites of Mizoram. This species shows the widest distribution among the four species (Fig. 4.2 & Table 1).

2. *Hylarana nicobariensis* (Stoliczka, 1870):

Diagnosis: Greyish or reddish brown above, uniform or spotted with darker; sides of head dark brown or black, which shade may be prolonged on the sides of the body below the lateral folds; a white streak on the upper lip; limbs with dark cross-bands (Fig. 4.20a&b). Vomerine teeth in oblique groups or short series between the chaonae, or extending a little beyond the level of their posterior borders; equally distant from each other or a little nearer the latter.

Description: Snout-vent length (SVL) of males ranges from 32.34 mm - 48.48 mm and females 42.36 mm - 58.6 mm (Table 3). Head longer than broad, much depressed; snout more or less projecting beyond the mouth, longer than the eye; canthus rostralis strong; loreal region nearly vertical, deeply concave; nostril nearer the tip of the snout than the eye; distance between the nostrils equal to the interorbital width, which equals or a little exceeds that of the upper eyelid; tympanum very distinct 3/5 to 4/5 the diameter of the eye, 3 to 5 times its distance from the latter. Prominent vomerine teeth present between chaonae (Fig. 4.21). Fingers long and slender, with a feeble dermal border, terminating in rather small discs which are as broad, $\frac{1}{2}$ to $\frac{2}{3}$ the diameter of tympanum, with the upper surface separated from the lower by a horse shoe-shaped groove; first finger longer than the second, third longer longest followed by the fourth finger; subarticular tubercles moderately large, very prominent (Fig. 4.22a). Hindlimb rather long and slender, the tibio-tarsal articulation reaching the nostril or the tip of the the snout, the heels strongly overlapping when the limbs are folded at right angles to the body; tibia 5 to $5\frac{1}{2}$ times as long as broad, $1\frac{1}{2}$ to 2 times in length from snout to vent, a little shorter than the forelimb, as long as or a little longer than the foot. Toes ending in a rather small discs, same as those of the fingers, $\frac{1}{2}$ to $\frac{2}{3}$ webbed, 2 or 3 phalanges of fourth free; the web rarely reaching the discs of the third and fifth toes; webbing formula: I_{1-1½}II₁₋₂III₁₋₂IV_{1½-1}V (Fig. 4.22b); outer metatarsals separated nearly to the base; subarticular tubercles rather small, prominent; no tarsal fold; inner metatarsal tubercle oval and outer tubercle rounded. Skin smooth or finely granulate above, with or without small warts; a strong, narrow or moderately broad glandular dorso-

lateral fold from above the tympanum to the hip. Lower parts smooth, posterior part of thighs granulated.

Sexual dimorphism: Males smaller than females. Males with internal vocal sacs; forelimb strong with a flat oval gland on the inner side of the arm and a moderately strong pad on the inner side of the first finger; throat and breast darker in the males.

Tadpole description:

Diagnosis: Body olive to gray-brown with loosely scattered black spots, tail muscle and fins mostly black with small, creamy patches, dorsal tail fin higher than ventral one, transparent marginal papillae.

Morphology: Body ovoid, snout rounded, slightly depressed; eyes dorsolateral, nostrils dorsal, open, equidistant between snout tip than eye; spiracle sinistral, extended as a short tapered tube, inner wall free from body, spiracular opening directed posterio-dorsally; medial vent tube with lateral displacement towards dextral, continuous with ventral fin. Tail lanceolate, dorsal fin margin weakly convex, both margins tapering abruptly at distal quarter towards a narrowly pointed tip. Dorsal fin originating at body-tail junction, gently sloping towards mid-tail convex, dorsal fin slightly deeper than ventral. Naso-lacrymal groove present.

Colour/Markings: Dorsum and flanks olive to gray-brown with scattered black spots, tail muscle and fins mostly black with small, creamy patches; venters unpigmented.

Oral Disc: Mouth ventral, sub-terminal, marginal papillae of anterior labium confined to lateral corners, consisting of three to four simple, triangular fleshy extensions on each side; marginal papillae of posterior labium continuous, consisting of transparent, elongated, tongue like extensions; short, bulbous

inframarginal papillae present; both jaw sheaths serrated at edge, keratinised at margins; LTRF: 2(2)/3(1).

Habit and Habitat: From the present observation, *Hylarana nicobariensis* is a nocturnal riparian species that frequents the vicinities of torrential or slow-flowing streams, rivers and rapids which are scattered with boulders of various sizes. They retreat in crevices between boulders, soils, dead logs and leaf litters during the daytime, and are active at night, perching on boulders or rocks, among pebbles in the vicinity of water bodies or sometime even in the water.

Distribution in Mizoram: *Hylarana nicobariensis* is widely distributed in Mizoram and was collected from the 27 surveyed sites of Mizoram except from Khawzawl, Lun dil and Khawhai (Champhai district), and Ramlaitui (Lunglei district) (Fig. 4.2&Table 1).

3. *Kaloula pulchra* Gray, 1831

Diagnosis: A stout-bodied, brightly-colored frog with short limbs and pointed head (Fig. 4.40). A dark triangular spot occupying the whole back from the middle of the eyelids and a lateral streak of the same color from the posterior corner of the eye, the two being separated by a yellow dorso-lateral stripe. Lower surfaces dark brown, mottled with light grey and cream colours; throat of the male infuscate.

Description: Snout-vent length (SVL) of males ranges from 57.74 mm - 69.76 mm and females 59.90 mm - 69.94 mm (Table 4). Head broader than long; snout short, round; canthus rostralis rounded; loreal region oblique; interorbital space 1½ to twice as broad as the upper eyelid; tympanum hidden. Vomerine teeth absent (Fig. 4.41). Fingers with small truncate dilatations distally; length of fingers 1<2<

$4 < 3$, the third finger longest and much longer than snout (Fig. 4.42a). Hindlimb rather short, mottled with brown, light grey and cream colours; toes short, scarcely dilated, with a rudiment of web; subarticular well developed (Fig. 4.42b); tibiotarsal articulation reaching the shoulder; heels do not overlap when hindlimbs folded at right angle to the body. Both inner and outer metatarsal tubercles present. Skin smooth or with irregular flat warts above; an indefinite fold from the eye to the forearm and sometimes one across the occiput. Lower surfaces smooth or faintly granular.

Sexual dimorphism: Males have darker throats than females. Females are generally larger than males.

Tadpole description:

Diagnosis: Body light to dark-brown with scattered black spots up to the level between eyes, tail muscle mostly black with small, creamy streak, tail fins more or less transparent with dark patches.

Morphology: Body oval, snout blunted, depressed; eyes dorsolateral, nostrils dorsal, not open, equidistant between snout tip than eye; spiracle posterior mid-ventral, extended as a short tapered tube, inner wall free from body, spiracular opening directed posterio-ventrally; medial vent tube continuous with ventral fin. Tail lanceolate, dorsal fin margin weakly convex, both margins tapering abruptly at distal $\frac{1}{3}$ towards a narrowly pointed tip. Dorsal fin originating at body-tail junction, slightly deeper than ventral. Naso-lacrymal grooves present.

Colour/Markings: Dorsum and flanks light to dark-brown with scattered black spots, tail muscle mostly black with small streak, and tail fins creamy with dark patches; venters pigmented with dark patches.

Oral Disc: Mouth terminal with dorsal and ventral semicircular labial flaps, no marginal papillae; both jaw sheaths and keratinised tooth row absent and no LTRF.

Habit and Habitat: *Kaloula pulchra* was found to be a nocturnal fossorial frog. They hide under leaf litter during the day hours and eat in the evening. For much of the time, the frogs stay out of sight by digging backward with their hind limbs into underground burrows, into piles of trash, and into other secretive spots they find along the ground. Breeding mainly takes place in temporary pools. In the present survey, this species was collected mostly from streams, paddy fields and ponds around anthropogenic area during the months of February to May during the years 2005, 2006 and 2007.

Distribution in Mizoram: In the present survey, *Kaloula pulchra* was collected from sixteen surveyed sites Aizawl, Rungdil, Sairang, Sihmu, Lengpui, Champhai, Khawzawl, Bilkawthlir, Kawnpui, Tuitun, Buhchang, Lawngtlai, Ramlaitui, Zawlnuam, Mat and Thenzawl (Fig. 4.2&Table 1).

4. *Microhyla berdmorei* (Blyth, 1856)

Diagnosis: Dorsally varying from pink to dark brown, sometimes with brownish spots or marbling scattered on dorsal surface of body (Fig. 4.60a&b). Characteristics sheds present dorsally in between the eyes, running up to trunk region; occasionally few minute brown spots also present on lateral parts of hindlimbs as well as on flanks. A chevron-shaped black marking present near anus. Both throat and chest mottled with dark brown. Limbs with faint cross bands. Dorsum of body generally smooth. Skin smooth; occasionally with few indistinct tubercles on back and on the sides of the body. Sometime dorsal skin is very loose. Ventrally smooth.

Description: A small frog, snout-vent length (SVL) of males 28.55 mm - 33.88 mm and females 30.68 mm - 35.7 mm (Table 5). Head much broader than length, slightly depressed; snout pointed, a little longer than eyes, projecting slightly beyond lower jaw; canthus rostralis prominent; nostrils a little closer to tip of snout than to eyes; tympanum hidden. Vomerine teeth absent (Fig. 4.61). Forelimbs moderately long; fingers slender, free with rounded tips; length of fingers $1 < 2 < 4 < 3$, the third finger longest and much longer than snout; subarticular tubercles large and prominent (Fig. 4.62a). Hindlimbs very long; tibiotarsal articulation reaching beyond tip of snout; heels strongly overlapping when hindlimbs folded at right angles to body; tibia $3\frac{1}{2}$ to 4 times as long as broad, more than $\frac{2}{3}$ the length of snout to vent; tips of toes swollen into rounded tips which are slightly larger than finger tips; fully webbed, web reaching near 4th phalanges of 4th toe, narrow but connecting tips with webbing formula: I₀₋₀II₀₋₀III₀₋₀IV₀₋₀V (Fig. 4.62b); inner metatarsal tubercle prominent and oval which is nearly $\frac{2}{3}$ of first finger; a small rounded outer metatarsal tubercle present.

Sexual dimorphism: Males have darker venter and a strong fold is present across the neck. Females generally being larger than males.

Tadpole description:

Diagnosis: Delicate; body light creamy to dark-brown with scattered black spots that forms a thick stripe up to the level between eyes, tail muscle mostly creamy and transparent, tail fins very delicate and transparent.

Morphology: Body ovoid, snout blunted, depressed; eyes dorsolateral, nostrils not open, dorsal, equidistant between snout tip than eye; spiracle posterior mid-ventral, extended as a very short tapered tube, inner wall free from body, spiracular opening

directed posterio-ventrally; medial vent tube continuous with ventral fin. Tail lanceolate, dorsal fin margin weakly convex, both margins tapering smoothly towards a narrowly pointed tip. Dorsal fin originating at body-tail junction, slightly deeper than ventral. Naso-lacrymal grooves present.

Colour/Markings: Dorsum and flanks light creamy at the anterior part, but dark-brown to black at the posterior part; venters unpigmented and transparent where the coiled intestine is visible.

Oral Disc: Mouth terminal with dorsal and ventral semicircular labial flaps, U-shaped medial notch protruded between the two flaps; no marginal papillae; both jaw sheaths and keratinised tooth row absent, therefore no LTRF.

Habit and Habitat: *Microhyla berdmorei* was found to inhabit various types of moist evergreen forest generally associated with hilly regions; also occurs in secondary growth, near the vicinity of water bodies. Breeding mainly takes place in standing waters. During their breeding season, they were commonly encountered around their breeding sites both day time and night time. During rainy season (April to September), they were found to retreat under forest litters and in the holes and crevices of soils and rocks. In the present study, this species was collected mostly during the months of October to March during the years.

Distribution in Mizoram: In the present study, *Microhyla berdmorei* was collected from twenty one surveyed sites Aizawl, Sairang, Sihmu, Lengpui, Durlui, Tuivawl, Tuirial, Tuirini, Tuichang, Champhai, Bilkawthlir, Tuitun, Buhchang, Lawngtla, Ramlaitui, Theiriat, Tut, Zawlnuam, Khankawn, Mat and Thenzawl. (Fig. 4.2 & Table 1).

II. Breeding behavior, habit and habitat, and ecological factors of the breeding sites:

1. Description of the breeding sites: After surveying the different parts of Mizoram, five locations/areas designated as study sites I, II, III, IV and V were selected which were observed and identified as good breeding grounds of the selected species. Locations of the study sites are as shown in Fig. 3.1. To understand the breeding behaviour of selected anurans, i.e., *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* the study sites were monitored during the years 2005, 2006 and 2007. The characteristic features of the study sites were as follows:

Study site I: It is located at Kawnpui town area (N 23° 58' 15.5"; E 92° 41' 30.9") at the elevation of ca. 310 m asl (Fig. 3.2). It consists of three permanent ponds measuring about 25 - 1369 sq.m in an area with an average depth of about 95 cm (Fig. 3.3a-c). *Nymphoides indicum* and *Drymaria cordata* are the main aquatic vegetation in this water body. About 10 m away from this pond, a stream named as Herhse is flowing in the direction of east to west. This study site was used for monitoring the breeding behavior and development of *Euphlyctis cyanophlyctis*.

Study site II: It is a section of Tuitun stream which runs from north to south direction. The study area lies between 23° 58' 21.27" – 40.19" N and 92° 41' 05.51" – 10.35" E (Fig. 3.4). The elevations range from 300 m to 325 m asl. The river gradient is steep and the width and depth vary considerably, related to rainfall patterns, but on average the river is about 8 m - 15 m wide and 50 cm - 360 cm deep in the center (Fig. 3.5a&b). The river has a permanent flow through alternating riffles, steep waterfalls and pools over bottoms of sand, gravel, huge boulders and

bed rock. The river banks consist of sand, mud, gravel, boulders and rock faces (Fig. 4.24a). This study site was used for monitoring *Hylarana nicobarensis* and *Microhyla berdmorei*.

Study sites I and II are situated about 60 km to the north from Aizawl, in the area of Kawnpui, Kolasib District. The area is dominated by shrubby vegetation like *Ageratum conyzoides*, *Bidens biternata*, *Crassocephalum crepidioides*, *Osbeckia crinata*, *Eupatorium riparium*, *Colocasia* sps., *Heydichium* sp., *Pterris* sps., *Mussauenda glabra*, *Spilanthes acmella*, *Thysanolaena maxima*, *Chromolaena odorata*, etc., bamboos are *Dendrocalamus* sps., *Neohouzeua dulloa*, *Bambusa tulda*, etc. and trees are *Careya arborea*, *Shorea robusta*, *Tectona grandis*, *Duabanga grandiflora*, *Mesua ferrea*, *Michelia champaca*, *Schima wallichii*, etc. Remaining forest areas are subject to varying levels of human disturbance from selective timber cutting, jhumming cultivation, rattan harvesting and hunting.

Study site III: This study side is located in the $23^{\circ} 47' - 48'$ N and $92^{\circ} 38' - 39'$ E; ca. 180 m - 184 m asl (Fig. 3.6). It includes rock-pools in the bed of the intermittent Sihhmui stream. These temporary rock-pools are completely or incompletely isolated from the main stream which is one of the tributaries of Tlawng river. The stream bed is rocky, intermittent, shallow or temporarily deep in some areas. The circumferences of the pools range from 97 cm to 325 cm with about 10 cm to 45 cm in depth, bottom contained a thin sandy soil with fallen leaves and twigs forming shelters for tadpoles (Fig. 3.7a&b). The breeding behavior and development of *Kaloula pulchra* was monitored at the study site III.

Study site IV: It lies in the $23^{\circ} 48' 24.66''$ N - $55.04''$ N and $92^{\circ} 38' 44.51'' - 39' 08.97''$ E along the stretch of Tlawng river, the longest river in Mizoram (Fig. 3.8 &

Fig. 3.9). The altitudes range from 35 m to 50 m asl. The habitat of this area until fairly recently consisted of mixed semi-evergreen and bamboo forest. Most of this habitat has now been cleared by anthropogenic activities such as teak (*Tectona grandis*) plantation, logging and jhum farming. However some isolated patches still remain. The breeding and development of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis* and *Microhyla berdmorei* were monitored from this study site.

Study site V: This study site is located in the 23° 44.144' N and 92° 40.282' E at the altitude of 865 m asl inside the campus of Mizoram University, Tanhril, Aizawl (Fig. 3.10). It consists of temporary pools and small rock-pools surrounded by a secondary forest (Fig. 3.11a&b). The pools are about 250 cm – 360 cm in diameter, whereas those rock-pools measured about 30 cm - 75 cm in diameter. It was used for monitoring the breeding behavior and development of *Kaloula pulchra*.

The study sites III, IV and V are located in the Aizawl district and the flora are, *Osbeckia crinata*, *Pterris* sps., *Centella asiatica*, *Ageratum conyzoides*, *Chromolaena odorata*, *Colocasia* sps., *Mikania micrantha*, *Mimosa pudica*, *Colona floribunda*, *Erythrina variegata*, *Bambusa tulda*, *Melocanna baccifera*, *Michelia champaca*, *Schima wallichii*, *Stereospermum colais*, *Tectona grandis*, etc.

During the course of studies it was found that these five study sites served as good habitat for breeding and development of both ranids (*Hylarana nicobariensis* and *Euphlyctis cyanophlyctis*) and microhylids (*Kaloula pulchra* and *Microhyla berdmorei*). Study sites II and IV are used for studying *Hylarana nicobariensis*; study sites I and IV for *Euphlyctis cyanophlyctis*; study sites II and IV for *Microhyla berdmorei* and study sites III and V for *Kaloula pulchra*.

2. Breeding behavior:

(i.) Breeding behavior of *Euphlyctis cyanophlyctis*: During the present investigation it is observed that *Euphlyctis cyanophlyctis* in Mizoram is a seasonal breeder and its breeding activity coincides with monsoon season i.e. March and continued up to the month of August. The study was conducted both in study site I which include three ponds (Fig. 3.3a-c & 4.6) and among the ephemeral standing pools along the river of Tlawng at study site IV (Fig. 3.9 and 4.7).

Courtship, mating calls and spawning: It was observed that *Euphlyctis cyanophlyctis* does not show reproductive behavior in both the study sites I and IV during the cold winter months from November to January with air temperature ranging from 10.5°C to 29°C (Table 6 & 12; Fig. 4.15), water temperature 11°C – 25.5°C (Table 7 & 13; Fig. 4.18), pH 6.4 to 7.4 (Table 7 & 13; Fig. 4.19), rainfall ranged from 0 to 69 mm and relative humidity between 41% to 92% (Table 6 & 12; Fig. 4.16 & 4.17). During this period, *Euphlyctis cyanophlyctis* was quite rare in the study sites. Following the first shower in the late-February or March where the atmospheric temperature ranged from 10.5°C to 29°C, relative humidity 41% - 87% and rainfall between 0 mm – 57.1 mm (Table 6 & 12; Fig.4.15, 4.16 & 4.17), water temperature between 11°C - 25°C, pH of water 7.1 - 7.3 (Table 7 & 13; Fig. 4.18 & 4.19), the calls of males were heard intermittently. During day time, both male and female were seen basking in the sun. Resting places surrounded by water are preferred to those on the shore. The adults are largely aquatic, floating motionless amongst plants with the lower half of body and hind limbs submerged in water. The skipper frog dives under water when disturbed, often staying beneath for long

durations. Call was very typical and infrequently heard almost throughout the year in the study fields, but showed a peak of activity during the breeding season starting from the month of March following increase in air temperature ($14^{\circ}\text{C} - 36^{\circ}\text{C}$), relative humidity between 43% and 87% and rainfall 1.2 mm – 228.42 mm (Table 6 & 12 and Fig. 4.15, 4.16 & 4.17); water temperature ($17^{\circ}\text{C} - 28^{\circ}\text{C}$) and pH 6.4 - 7.2 (Table 7 & 13; Fig. 4.18 & 4.19). The males started calling from 3:30 PM and were found croaking by floating on the surface of water among aquatic plants in the breeding sites (Fig. 4.8a&b). While calling from water, the external vocal sacs were visible on the sides of the throat. Males would call sporadically during the day and in chorus from dusk onwards.

Advertisement calls were emitted in series with variable call intervals. The call lasted for about 524.5 - 683.4 ms (Mean \pm SD = 599.7 ± 55.16 , n=10) emitted at an interval of 0.802 - 1.650 s (Mean \pm SD = 1.302 ± 0.355 , n = 10), each call is composed of 5-12 pulses. Each pulse lasted for 18 – 56.125 ms (Mean \pm SD = 31.854 ± 11.932). The amplitude of the note increased after one fourth ($\frac{1}{4}$), then decreased quickly and again increased in its last third fourth ($\frac{3}{4}$). The frequency spectra ranged between 1140 – 1609 Hz (Fig. 4.9). Rarely individual calling males were observed at night. This observation showed that these aquatic frogs having definite territorial habitat, generally breed in territorial breeding sites in the lentic pools filled with dense vegetations. After making a continuous call made by males (i.e. around 8:30 PM), the female already inside the pond, on hearing the advertisement call of a particular male of the breeding area, primarily selects him and swims towards the same calling male. After confirming her selection of mate, the female move towards the front of the male. Intermittent calls were also observed

during this particular time, the male may resume calling again with high posture on the water surface. Within a few minutes, it was found that the calling male readily clasped the female which was followed by the axillary amplexus. Pairs in amplexus can be seen in water surfacing and submerging together. Breeding usually took place in the littoral region i.e. in the vicinity of the bank of the water (Fig. 4.10a&b). During field observations, it was documented that the males who were not taking part in the amplexus were heard to continue calling till 4:30 AM of that following day.

Amplexus lasted for about 2 hours or even up to 4 hours or sometimes even longer, and then the female deposited eggs as a slimy mass amidst aquatic plants like *Drymaria cordata* and *Nymphoides indicum* in the study site I, but in the study site III the eggs were found to attach along with algae (*Cladophora* sp.) or scattered among the grasses at the edge of muddy pools (Fig. 4.12). No parental care was observed among them, as soon as the eggs were laid both male and female moved away from the oviposition site in the field as well as in captivity (Fig. 4.11). Spawning started only from the month of March and continued till August where air temperature recorded was 18 °C – 33 °C, relative humidity between 44.5% - 97% and rainfall between 266 mm – 600.1 mm during the study period (2005 - 2007) as shown in the Table 6 & 12 and Fig. 4.15, 4.16 & 4.17; water temperature 19.5°C – 29°C, pH of water 6.5 - 7.1 (Table 7 & 13; Fig. 4.18 & 4.19). Usually from the month of September, following the decreased in air temperature 21 °C - 35.5°C, water temperature 20°C – 27°C, pH 6.5 - 7.4, rainfall (214.4 mm - 611.4 mm) and relative humidity 42% - 94% (Table 6, 7, 12 & 13; Fig. 4.15, 4.16, 4.17, 4.18 & 4.19), although males vocalizations were irregularly heard, breeding activity was

not seen from this month. However, adults and sub-adults were not uncommonly encountered in the fields till November.

ANOVA tests on the difference of significant (at 0.05 level) between the breeding season and environmental factors (i.e. air temperature, relative humidity, rainfall, water temperature and pH of water) at study site-I were $p=0.000$, $p=0.980$, $p=0.000$, $p=0.006$ and $p=0.604$ while at study site-IV, $p=0.064$, $p=0.751$, $p=0.002$, $p=0.014$ and $p=0.754$, respectively (Table 16a-e & Table 17a-e).

In the year 2005, 2006 and 2007 the number of egg masses recorded during the breeding period i.e., from the months of March to August at study site I was 13, 9 and 8 and at study site IV was 7, 11 and 12 respectively.

Size of amplexing pairs and clutch size: In the year 2005, twenty amplexing pairs along with their eggs were collected from the breeding sites and their snout-vent length and the clutch sizes were recorded. In order to know the clutch size, the above twenty numbers of egg clutches recorded in the year 2005 were collected and brought to the laboratory. The eggs were counted and kept in a tray containing pond water for further development. Similarly, collections were also made in the years 2006 and 2007 (Table 24). The clutch sizes recorded ranged from 68 – 182 (Mean $\pm SE = 136.73 \pm 4.16$). No correlation was found between the sizes of females and clutch sizes ($r = 0.172$; $p = 0.189$). Distinct sexual size dimorphism was observed in this species where the females were larger (Mean $\pm SE = 51.69 \pm 0.86$; range = 31.23 mm - 61.15 mm; $n=60$) than the males (Mean $\pm SE = 39.6 \pm 0.38$; range = 32.20 mm - 46.80 mm; $n=60$), and male are with external vocal slits. The frogs were released back after recording the morphometric measurements.

Histology of testis and ovary of *Euphlyctis cyanophlyctis*: Testis and ovary of *Euphlyctis cyanophlyctis* were studied and observed histologically during the breeding season. The testis of *Euphlyctis cyanophlyctis* weighed between 0.004 gm – 0.018 gm. During the breeding season, the histological sections of the testis of *Euphlyctis cyanophlyctis* observed showed the presence of different stages of spermatogenic cells and sertoli cells. Spermatogonia are spherical in shape and uniformly distributed around the periphery of the seminiferous tubule. Spermatocytes can be classified into primary and secondary. Primary spermatocytes are irregularly oval or spherical in shape, possessing large and vesicular nuclei, and they are larger than the spermatogonia. Secondary spermatocytes are oval in shape and are smaller than the primary spermatocytes, and are lie toward the lumen of the tubule. Numerous spermatids are also more concentrated around the lumen. The tubule was filled with slender and filamentous spermatozoa. Mature spermatozoa occur in bundles (Fig. 4.13).

During the breeding period the weight of the ovaries ranged between 0.81 gm and 1.38 gm. The sections of the ovary during the breeding period showed the presence of outer peritoneal covering, the theca externa and inner covering, the theca interna, and growing as well as mature oocytes (Fig. 4.14). The theca interna surrounds each egg except for the limited area bulging toward the body cavity, where it is covered by only the theca externa. Each mature oocyte was found to be covered by a transparent vitelline membrane and had large amount of yolk distributed in the cytoplasm.

(ii.) Breeding behavior of *Hylarana nicobariensis*: Observations on the breeding behavior of *Hylarana nicobariensis* was conducted at Tuitun stream, study site II

and Tlawng river, study site IV located at Kawnpui and Sairang respectively (Fig. 3.4, 3.5a&b, 3.8, 3.9 and 4.24a&b). The availability of *Hylarana nicobariensis* in the fields was documented during October to April in the main water and its surroundings.

Courtship, mating calls and spawning: Field observations indicated that *Hylarana nicobariensis* is a seasonal stream-breeder and the breeding takes place from October to February, sometimes even up to March depending on the timing of monsoon which coincide with late autumn to winter season in Mizoram. During the breeding season (October - February), the water became shallow and slow flowing (Figs. 4.24a&b) and it was observed to occur within a range of air temperature between 10°C and 35°C, rainfall 0 mm - 269.3 mm, and relative humidity 42% - 96% (Table 8 & 12; Fig. 4.35, 4.36 & 4.37), water temperature 12°C - 24.5°C and pH 6.5 - 7.4 (Table 9 & 13; Fig. 4.38 & 4.39), and the peak period of abundance is December when the water become very shallow with minimum velocity, and the ambient air temperature between 11.5°C - 29°C, relative humidity of 42 – 91%, and no rain fall at all (Table 8 & 12; Fig. 4.35, 4.36 & 4.37); water temperature 14.5°C - 19.5°C, pH 6.4-7.2 (Table 9 & 13; Fig. 4.38 & 4.39). The frogs were observed in cracks of soil and boulders, in pebbles or holes of dead logs, partially submerged in water; few were also located floating on water.

Male mating vocalization was heard from cracks of soil and rock near the bank, pebbles and boulders near water, from holes of dead logs, twigs and leaves partially submerged in water, by floating among aquatic plants, or from shallow water (Fig. 4.25a-e). The males call mainly during night beginning at 4:30 PM and unsuccessful males continued until the following day (6:00 AM). The call consisted

of 5 - 9 notes (Fig. 4.26) emitted at an interval of 0. 628 - 1.645s, each note ranged from 0.141-0.165 s with a series of 35 to 47 pulses. A single call lasted for about 1.232 – 2.682 s. The amplitude of the note increased its second to third fourth then decreased slowly until the end, as did the amplitude of the pulses. The frequency spectra ranged between 2109 – 2296 Hz with a dominant band at 2265 Hz.

In response to the mating call, females came out from their hides among the surrounding vegetations and approached the breeding ground (Fig. 4.27). Within a short period amplexus takes place. The amplexus was axillary and amplexing pairs floats on water or perch on the substrate until eggs are laid (Figs. 4.28a-e). However, mating congregation and combat between the males were not seen in this frog; rather, they prefer solitary places for breeding. Occasionally, amplexing pairs were also encountered among leaf litters even up to 150 m away from the breeding ground Fig. 4.28c). Amplexus (duration: 1 – 6 hours) and egg laying (duration 10 – 20 minutes) occurred both day and night. The eggs are laid in one clutch, which remains submerged and attached to a substratum in water about 5 cm – 10 cm, such as rocks, pebbles, aquatic plants, dead logs, even among other laid egg clutch etc.(Fig. 4.30 & Fig. 4.31a-c). Sometimes, more than one clutch of eggs (even up to 27 clutches) were seen in the communal egg masses adhered with each other on the substrata in the same oviposition site along the rivers or streams (Fig. 32a&b). After laying clutch of eggs, both the male and female move away from the breeding site and the eggs are left to develop on their own (Figs. 4.29a&b). The egg was with animal pole, vary from 1.2 mm – 1.4 mm in diameter. Field observations in the present study revealed that during the non-breeding season as the river was flooded (Figs. 4.23), the frogs move up to the woodland and could only be traced in moist

leaf litter in the forested area near the river which was about a hundred meters away from the breeding ground. During this period (i.e., March to September), air temperature ranged from 14°C - 35.5°C, rain fall from 1.2 mm -704 mm and relative humidity 43%-98% (Table 8 & 12; Fig. 4.35, 4.36 & 4.37), water temperature 17°C – 29°C, and pH fluctuates between 6.4 – 7.6 (Table 9 & 13; Fig.4.38 & 4.39). During the months of March and April although tadpoles, sub-adult and some adult of *Hylarana nicobarensis* were encountered, no sign of breeding behavior was observed and the animal start to retreat in the forest area from the water bodies. Then following the continuation of rainfall, during the period between May to August where the average monthly rainfall was 403 mm±30.44 (121 mm – 704 mm) with the humidity fluctuates between 47% and 96% and average atmospheric temperature was about 15.5°C - 33°C (Table 8 & 12; Fig. 4.35, 4.36 & 4.37); water temperature 18°C – 28.5°C and pH of water between 6.4 – 7.6 (Table 9 & 13; Fig. 4.38 & 4.39), *Hylarana nicobarensis* were very rare and few were found among the forest litters.

At 0.05 level of significant, ANOVA tests on the difference of significant between the breeding season and environmental factors (i.e. air temperature, relative humidity, rainfall, water temperature and pH of water) at study site-II were p=0.027, p=0.000, p=0.000, p=0.001 and p=0.046, while at study site-IV, p=0.000, p=0.002, p=0.002, p=0.000 and p=0.535, respectively (Table 18a-e & Table 19a-e).

Size of amplexing pairs and clutch size: In the year 2005, 2006 and 2007, the number of amplexing pairs along with their clutch sizes recorded during the breeding period i.e., from the months of January-February, and October-December at study site II was 8, 5 and 7 and at study site IV was 12, 15 and 13, respectively.

In the years 2005, twenty amplexing pairs along with their eggs were collected from the breeding sites, and their morphometric measurements and the clutch sizes were recorded. Similarly, collections were also made in the years 2006 and 2007 (Table 25). The clutch sizes recorded ranged from 131 – 628 (Mean \pm SE= 405.62 \pm 22.75). No correlation was, however, observed between female body size and clutch size ($r = 0.230$; $p = 0.076$). Distinct sexual size dimorphism was observed in this species where females were larger (Mean \pm SE= 51.56 \pm 0.55; range= 42. 36 - 58.6 mm; n=60) than males (Mean \pm SE= 39.06 \pm 0.51; range= 32.34 - 48.48mm; n=60), and males are with darker throat and oval flat gland on the inner arm. The frogs were released back after recording the measurements.

Histology of testis and ovary of *Hylarana nicobariensis*: Testes and ovary of *Hylarana nicobariensis* were studied and observed histologically during the breeding period. The testis of *Hylarana nicobariensis* weighed between 0.008 gm – 0.024 gm. The histological section of testis showed the presence of spermatozoa in the seminiferous tubule which indicated the beginning of the breeding phase. During this phase, the seminiferous tubules were more or less rounded in shape. Different stages of spermatocytes, spermatogonia (SG), primary spermatocytes (PS), secondary spermatocytes (SS), spermatids (ST) and spermatozoa (S), and sertoli cells are observed. Interstitial tissue was well developed and the tubule was filled with numerous spermatozoa. Spermatozoa were observed to be slender and filamentous. Bundles of mature spermatozoa occur in a cluster with their tail extending into the lumen of the tubules (Fig. 4.33).

During the breeding period the weight of the ovaries ranged between 0.746 gm and 1.254 gm. The sections of the ovary during the breeding period showed the

presence of growing oocytes as well as primary oocytes (Fig. 4.34). The outer membrane, theca externa and the inner membrane, theca interna were also observed. Each mature oocyte was found to be covered by a vitelline membrane and had yolk platelets scattered in the cytoplasm.

(iii.) Breeding behavior of *Kaloula pulchra*: During the study period, it was observed that *Kaloula pulchra* was a seasonal breeder and its breeding activity coincides with the onset of monsoon i.e. late February to May in Mizoram. The study was conducted for three years (2005 to 2007) among rock-pools found on the bed of forest-edge stream at Sihhmu, study site III (Fig. 3.6, 3.7a&b and 4.43a&b) and in the ephemeral standing pools and the adjacent rock-pools surrounded by secondary forested area at study site V in the Mizoram University Campus (Fig. 3.10, 3.11a&b and 4.44a&b). The bottoms of all the pools were filled with leaf litters and humus that provided shelters for tadpoles.

Courtship, mating calls and spawning: In the present observation, the breeding activity of *Kaloula pulchra* was stimulated by the first shower of monsoon rain in Mizoram. When the first shower came in the month of late February except in the year 2006 (where the first shower started only from March), it was observed that the frogs came out from their hibernation and started to call from the wetter part of the study sites. During the early part of February, the atmospheric temperature ranged between 14°C to 28.5°C and relative humidity 42% - 86% (Table 10 & 14; Fig. 4.55 & 4.57), the breeding pools were dried up except in the year 2007 in which only the pool in the study site V was filled with small amount of water, where water temperature fluctuates from 14.5°C to 26°C, pH 6.1 (Table 15; Figs. 4.58 & 4.59),

rainfall only between 0 mm – 39.1 mm and relative humidity between 48% - 86% (Table 14; Figs.4.56 & 4.57) during the years 2005, 2006 and 2007 in study sites III and V. Within 2 – 3 weeks of the first shower when enough water was available in the breeding place, normally from the months of late February to May the frogs came out to mate in the pools that were filled with water, where the atmospheric temperature ranged between 14°C to 33°C, rainfall between 3 mm - 539.7 mm and relative humidity fluctuates between 47% - 92% (Table 10 & 14; Figs.4.55, 4.56 & 4.57), water temperature 14.5°C to 28.5°C , pH between 6.2 - 6.7 (Table 11 & 15; Figs.4.58 & 4.59), during the years 2005, 2006 and 2007 in study sites III and V. At the onset of dusk, the males first enter the breeding site and float in the pools, and blow up their bodies to make calls (Fig. 4.45a&b). Their sounds are loud and deep like a bull moo. Their rounded shape is exaggerated even more when they inflate while floating and calling. Advertisement calls were emitted in series with variable call intervals. The call consisted of a single note (Fig. 4.46) emitted at an interval of 2.876 - 3.902 s (Mean \pm SD = 3.344 \pm 0.386, n = 10), the note repetition rate ranged from 0.21 - 0.25 notes per second (Mean \pm SD = 0.23 \pm 0.015, n = 10). The notes lasted 450.8-620.3 ms (Mean \pm SD = 527.8 \pm 65.671, n=10) and were composed of a series of 36 – 45 pulses. The amplitude of the note increased quickly in its second third, then decreased more slowly until the end, as did the amplitude of the pulses. The frequency spectra had a dominant band at 1265 Hz.

Male mating call started from 4:00 PM and a single male vocalization can be heard from a distance of about 100 m away. In this observation, the distance between two adjacent calling males in the breeding ground are usually 2 m – 10 m apart (Figs. 4.43a&b). A single male usually occupied one rock-pool, if more than

one male occupied a small rock-pool, the dominant male would drive out other males. In the present observation, the existence of territorial combat for the breeding ground among males *Kaloula pulchra* was documented. Callings was in chorus, however adjacent calling males alternated their calls. In this study vocalization of unsuccessful male was noticed till 6:00 AM in the next morning. During observation in the fields females were encountered in the forest-edge approaching the breeding ground in response to the calls. Normally, after 2 – 5 hrs of continuous calling, females arrived in the breeding ground and mating was usually observed from 18:00 PM to 12:00 AM. After entering the breeding ground, the female approached the calling male from the back and with the help of her fore-limbs she started tackling the hind-limbs and flanks of the calling male. Suddenly, the male turned back and mounted on the back of the female (Fig. 4.47a&b). Pairs in amplexus can be seen in water submerging together usually in the corner of pool (Fig. 4.49a&b). Amplexing was axillary and a single pair was usually found to occupy each water-hole in the study site-III, where as two to three pairs were also encountered in the larger pools as in the case of study site-V. In case of large breeding ground, a dominant male first mate with a female that first entered the pool while other males continued calling for other females (Fig. 4.48). Male-male combat for the female or sexual conflicts between male and female was not observed. Amplexing usually last for 20 minutes to 1 hr. Females laid eggs quickly which last for 10 minutes to 15 minutes. After laying eggs, the female was seen leaving the breeding site, while the male was found to stay for a longer period of time around the clutch of pigmented eggs ($1.48 \text{ mm} \pm 0.01$ in diameter) floating on the surface of water (Fig. 4.50 & Fig. 4.51). After some time the male also left the

eggs to hatch on their own (Fig. 4.52a&b). No parental care was documented in this species.

During the month of June, when the atmospheric temperature ranged between 15°C to 32°C, rainfall between 83.5 mm – 525 mm and relative humidity 50% - 98% (Table 10 & 14; Fig. 4.55, 4.56 & 4.57), water temperature 14.5°C to 28°C , pH between 6.5 - 7.2 (Table 11 & 15; Figs. 4.58 & 4.59), in study sites III and V, although a few calling males were occasionally encountered in the field, no amplexing pairs as well as egg clutches were recovered during this particular month. From the month of July to January, *Kaloula pulchra* were not found in the study areas and already retreated to their subterranean habitats in the surrounding forests where the atmospheric temperature fluctuated between 10°C – 35.5°C, rainfall from 0 mm – 612.1 mm and relative humidity 43% - 97% (Table 10 & 14; Fig. 4.55, 4.56 & 4.57), water temperature ranged between 11.5°C - 29°C and pH 6.1 - 7.3 (Table 11 & 15; Fig. 4.58 & 4.59), in study sites III and V.

ANOVA tests on the difference of significant (at 0.05 level) between the breeding season and environmental factors (i.e. air temperature, relative humidity, rainfall, water temperature and pH of water) at study site-III were p=0.604, p=0.458, p=0.375, p=0.087 and p=0.139, while at study site-V, p=0.598, p=0.094, p=0.603, p=0.828 and p=0.121, respectively (Table 20a-e & Table 21a-e).

Size of amplexing pairs and clutch size: *Kaloula pulchra* exhibited a distinct sexual size dimorphism where males are with darker throat and smaller (SVL= 61.92 ± 0.49 mm) than the females (SVL= 64.60 ± 0.51 mm) as shown on the Table 4.

In order to know the clutch size, 13 numbers of eggs masses along with amplexing pairs were collected from the field and brought to the laboratory in the

year 2005. Similarly, 17 and 20 numbers were collected in the years 2006 and 2007 respectively. The clutch size varied from 363 - 576 with a mean of 482.3 ± 7.37 in different clutches (Table 26). No correlation was found between the snout-vent length of females and clutch sizes ($r=0.187$; $p=0.167$). After taking the morphometric measurements of adults and tadpoles, some were preserved and the rests were released back to their natural environment

Histology of testis and ovary of *Kaloula pulchra*: Testis and ovary of *Kaloula pulchra* were also studied and observed histologically during the breeding season. Weight of the testis varied between 0.045 gm – 0.087 gm. The histological section of testis during the breeding season showed seminiferous tubules which were more or less oval in shape, and well developed sertoli cells were also observed. The lumen of seminiferous tubule was filled with bundles of spermatozoa. Seminiferous tubules were surrounded by epithelium made up of sertoli cells and spermatogonic cells. Spermatozoa were observed to be slender and filamentous. Other than spermatozoa, different stages of spermatocytes i.e., spermatogonia, primary spermatocytes, secondary spermatocytes and spermatids were also observed (Fig. 4.53).

During the breeding phase there are several lobes on each side of the ovary and its size varied depending on the maturity of the ova in the ovary (Fig. 4.54). The weight of the ovaries ranged between 1.676 gm - 2.531 gm during the breeding period. Both growing and mature oocytes were found to be present during the breeding phase and they were covered by external membrane (theca externa) and internal membrane (theca interna). Yolk platelets are found to be distributed throughout the cytoplasm.

(iv.) Breeding behavior of *Microhyla berdmorei*: The breeding behavior of *Microhyla berdmorei* was monitored along the stream of Tuitun, Study site-II (Fig. 3.4, 3.5a&b and 4.63), and Tlawng river, Study site-IV (Fig. 3.8, 3.9 and 4.64). In both the study sites, the breeding usually takes place in the standing pools, covers and mixes with vegetations and all the sites were mostly located at the edges of the main water bodies.

Courtship, mating calls and spawning: During the last part of monsoon season, i.e. late September when the rivers and streams started receding, *Microhyla berdmorei* were seen among leaf litter, bush, and crevices of rocks and soil around the stream and river where atmospheric air temperature ranged between 21°C – 35.5°C, rain fall from 214.4 mm – 611.4 mm and relative humidity 54% - 94% (Table 8 & 12; Fig. 4.73, 4.74&4.75), water temperature 20°C – 26.5°C, and pH fluctuates between 6.6 –7.5 (Table 9 & 13; Fig. 4.76&4.77). Males came out from their hides and locate suitable breeding sites and start calling in the month of October where atmospheric air temperature ranged between 16°C – 35°C, rain fall from 67 mm – 269 mm and relative humidity 48% - 96% (Table 8 & 12; Fig. 4.73, 4.74 & 4.75), water temperature 16.5°C – 24.5°C, and pH fluctuates between 6.5 – 6.9 (Table 9 & 13; Fig. 4.76&4.77). Male mating call started at 16:30 PM and unsuccessful males continued calling till next morning (6:30 AM). Single male intermitted calls which were audible from even a distance of 30 m. A large chorus of *Microhyla berdmorei* called intermittently from secluded spots under pebbles, debris of twigs and small shrubs, and fissures of soil in the breeding area (Fig.4.66a-d). Male calling site is usually at a distance of 30 cm to 150 cm from the water margin of the pool. The distance between the two adjacent calling males varies from

2 m – 5 m apart. The calling position was upright, the head held upwards with the help of stretched forelegs and the hind legs are folded (Fig. 4.66b-d). The call consisted of a single to seven notes emitted at an interval of 3 -5 s, a single call lasted for 1.4 -1.6 s (Fig. 4.67). Each note consisted of 7 – 12 pulses, lasted for 0.2 s at an interval of 0.06 s. The amplitude of the note increased slowly and reached the peak in the middle which then decreased slowly until the end. The frequency spectra ranged between 1484 Hz – 2046 Hz with dominant frequency 1677 Hz.

At around 8:00 hrs to 10:00 PM, the arrival of female at the pool occurred after chorus formation, contacted the male and they were typically observed in amplexus before midnight. The male clasped the female in axillary amplexus and was recorded either submerged or on the available substratum in the pool edge (Figs. 4.68a-d). Amplecting may usually last for 2 – 3 hrs or even longer periods. While in axillary amplexus, the male clasped the female and pressed her abdomen, which apparently facilitated egg deposition. During the peak of breeding period, i.e. November, male vocalization and amplecting pairs were also encountered in and around the breeding sites even during day time. After laying a floating raft of pigmented eggs, the female left the breeding site first then followed by the male (Figs. 4.69a&b), and no parental care was observed (Fig. 4.70a-c).

From the month of October, the breeding activity continued till March of the next year. During this period, the atmospheric air temperature ranged between 10°C – 33.5°C, rain fall from 0 mm - 269.3 mm and relative humidity 41% - 96% (Table 8 & 12; Fig. 4.73, 4.74 & 4.75); water temperature 12°C – 23.5°C, and pH fluctuates between 6.3 –7.4 (Table 9 & 13; Fig. 4.76 & 4.77). The breeding peak was observed in the month of November when the atmospheric air temperature ranged between

13.5°C – 32°C, rainfall from 0 mm – 69 mm and relative humidity 44% - 92% (Table 8 & 12; Fig. 4.73, 4.74 & 4.75), water temperature 16°C – 21.5°C, and pH fluctuates between 6.5 – 7.4 (Table 9 & 13; Fig. 4.76 & 4.77), Though there was no more breeding activity in the month of April and May, few were encountered in the surrounding forest areas of the study area. During the monsoon season (June to August), the streams and rivers were flooded and the species were very rare and not available in the breeding area as well as in the adjacent forest area, air temperature ranged between 15.5°C – 33°C, where rainfall from 121 mm – 704 mm and relative humidity 48% - 97% (Table 8 & 12; Fig. 4.73, 4.74 & 4.75), water temperature 18°C – 29°C, and pH fluctuates between 6.4 – 7.6 (Table 9 & 13; Fig. 4.76 & 4.77).

At 0.05 level of significant, ANOVA tests on the difference of significant between the breeding season and environmental factors (i.e., air temperature, relative humidity, rainfall, water temperature and pH of water) at study site-II were p=0.000, p=0.000, p=0.000, p=0.001 and p=0.783, while at study site-IV, p=0.000, p=0.000, p=0.000 and p=0.821, respectively (Table 22a-e & Table 23a-e).

Size of amplexing pairs and clutch size: In the years 2005, 2006 and 2007, the number of egg masses along with amplexing sizes recorded during the breeding period i.e., from the months of January-March, and October-December at study site II was 11, 13 and 12, and at study site IV was 9, 7 and 8. In the years 2005, twenty amplexing pairs along with their eggs were collected from the breeding sites and their morphometric measurements and the clutch sizes were recorded. Similarly, collections were also made during their breeding season in the years 2006 and 2007 (Table 27). Clutch sizes ranged between 218 – 443 (Mean \pm SE = 352.9 \pm 8.46). No correlation was observed between female body size and clutch size ($r = -0.132$; $p =$

0.314). No distinct sexual dimorphism was observed in this species except the females were slightly larger (Mean \pm SE= 33.9 \pm 0.16; range= 31. 02 - 34.35 mm; n=60) than the males (Mean \pm SE= 31.03 \pm 0.17; range= 28.45 - 33.88mm; n=60), and males were usually with darker venter. The frogs were released back after recording their morphometric measurements.

Histology of testis and ovary of *Microhyla berdmorei*: Histological studies on testis and ovary of *Microhyla berdmorei* were also conducted during the breeding season. The testis weighed between 0.004 gm – 0.006 gm. During the breeding season, the histological sections of the testis of *Microhyla berdmorei* showed the presence of different stages of spermatogenic cells and sertoli cells. During this phase seminiferous tubules were more or less rounded in shape and the tubules were filled with bundles of elongated spermatozoa. The peripheral region of the tubule was occupied by spermatogonia, followed by primary and secondary spermatocytes, spermatids and spermatozoa. Mature spermatozoa occur in a cluster with their tail extending into the lumen of the tubules. (Fig. 4.71).

During the breeding period the weight of the ovaries ranged between 0.724 gm and 1.356 gm. Sections of the ovary during the breeding period showed the presence of growing as well as mature oocytes (Fig. 4.72). Each mature oocyte was also found to be covered by a vitelline membrane and had a large amount of yolk platelets in the cytoplasm. Theca externa and theca interna were also observed.

III. Development and Metamorphosis:

Studies on the successive ontogenetic stages, to record the normal developmental table, are important in understanding the ecology of species and for planning conservation measures. Appropriate staging of the larval period is, therefore, fundamental to various life history studies of amphibians.

During the course of this study the developmental stages of the four species were recorded from the time of egg laying till the embryo hatched into a tadpole, and metamorphosis of the tadpole into a froglet at their corresponding study sites under natural environment, and the egg masses collected from the study sites were reared and monitored in the laboratory. The stages in the entire developmental series are selected on the basis of external morphological characteristics as described by Gosner (1960), and altogether 46 different developmental stages were recorded for each species. The measurements made in different stages include diameters of the eggs, sizes of early embryos and total lengths of larvae. For each stage, ten numbers were used for morphometric measurement.

1. Developmental Stages and Metamorphosis of *Euphlyctis cyanophlyctis*: The developmental stages of *Euphlyctis cyanophlyctis* were recorded from the time of egg laying till the completion of metamorphosis from March to August during the study period at the study sites I (water temperature 18 °C – 33 °C; pH= 6.5 - 7.2) and IV (water temperature 17 °C - 28.5 °C; pH= 6.4 - 7.6) under natural environment and in the laboratory at water temperature between 21°C and 30°C. The development and metamorphosis was therefore completed within about 64 – 65 days in *Euphlyctis cyanophlyctis* at temperature between 21° C and 30° C in the

natural environment. In the laboratory also, the pattern and duration of development and metamorphosis was observed to be more or less the same during the breeding season (Table 28). A brief account of various stages of development and metamorphosis of *Euphlyctis cyanophlyctis* was given in the following sections.

Stage 1 – Unfertilized egg: The freshly laid egg is spherical in shape with the animal hemisphere pigmented dark brown to greyish and the yolk vegetal hemisphere almost white. It measures about 1.35 ± 0.03 mm in diameter. It is surrounded by a thin, transparent, vitelline membrane. Around the egg is the jelly capsule which swells on contact with water (Fig. 4.78.1).

Stage 2 – Fertilized egg: A faintly pigmented zone, the gray crescent is visible on dorsal side of the egg. Animal hemisphere is dark brown and the vegetal hemisphere is whitish. It measures about 1.36 ± 0.05 mm. This stage is observed within 25 mins (Fig. 4.78.2).

Stage 3 – Two cell stage: A furrow appears in the animal hemisphere after fertilization. This furrow extends down through the vegetal hemisphere, dividing the egg into two blastomeres. The cleavage furrow passed through the gray crescent. It measures about 1.36 ± 0.06 mm. The first cleavage was completed in 1 hr 45 mins after fertilization (Fig. 4.78.3).

Stage 4 – Four cell stage: The second cleavage was also found to occur in the meridional plane at right angle to the first cleavage, and progressed from the animal pole and dividing the egg dividing it into four blastomeres. The complete four cell stage was observed at 2 hrs 20 mins after fertilization. The embryo measures 1.36 ± 0.03 mm (Fig. 4.78.4).

Stage 5 – Eight cell stage: The third cleavage is horizontal, slightly above the equator of the egg and at right angle to both the first and second cleavages. Eight blastomeres have formed. The four smaller micromeres of the animal pole are pigmented and the four bigger macromeres of the vegetal pole are unpigmented. Completion of the eight cell stage was recorded at 3 hrs 45 mins. The size remains more or less constant i.e. 1.36 ± 0.02 mm (Fig. 4.78.5).

Stage 6 – Sixteen cell stage: The fourth cleavage furrows are found to be vertical. Sixteen blastomeres are formed by division of each blastomere. The egg size slightly increased and measured 1.37 ± 0.04 mm. It is observed after 5 hrs and 15 mins. (Fig. 4.78.6).

Stage 7 – Thirty two cell stage: The fifth cleavage is horizontal and asynchronous. The cleavage furrow cut the micromere completely and equally but the furrow in the vegetal hemisphere divides the macromeres unequally resulting in the formation of sixteen smaller micromeres and sixteen larger macromeres. This stage took 6 hrs and 30 mins to complete and measures 1.36 ± 0.05 mm ((Fig. 4.78.7).

Stage 8 – Mid cleavage: The animal pole blastomeres i.e. micromeres divided equally and smaller in size, while the vegetal pole blastomeres i.e. macromeres divided unequally. The cells in the animal pole become smaller while the cells of the vegetal hemisphere remain larger. This stage is also known as morula, and measures about 1.36 ± 0.64 mm. in diameter. This stage indicates the beginning of blastulation. The mid cleavage stage took place after 8 hrs 30 mins (Fig. 4.78.8).

Stage 9 – Late cleavage: The number of blastomeres has further increased and the surface of the embryo looks granular. The micromeres are very minute in size. The

embryo is considered as blastula, and measures 1.38 ± 0.25 mm. It is observed at 10 hrs 15 mins (Fig. 4.78.9).

Stage 10 – *Dorsal lip*: The extension of the dorsal micromeres over the macromeres indicated the beginning of gastrulation. Due to invagination of the micromeres, a crescent shaped i.e. the dorsal lip of blastopore appeared slightly below the equator on the dorsal side of the embryo at 15 hrs 30 mins. The egg measured 1.38 ± 0.01 mm (Fig. 4.78.10).

Stage 11 – *Mid gastrula*: Continued epibolic migration of micromeres over the vegetal hemisphere has greatly reduced the exposed area of macromeres and constituted the yolk plug. It took about 21 hrs 15 mins and the gastrula measured 1.39 ± 0.02 mm (Fig. 4.78.11).

Stage 12 – *Late gastrula*: The small protruding blastopore gradually is reduced due to constriction of the blastoporal lips. The late gastrula measured 1.45 ± 0.06 mm. The whole process takes about 25 hrs 45 mins (or 1 day 1:45 hr) (Fig. 4.78.12).

Stage 13 – *Neural plate*: The embryo is slightly elongated and the dorsal surface has flattened to form the neural plate, and the lateral ridges became slightly elevated forming neural folds. The embryo measured 1.88 ± 0.06 mm. This stage is observed at 30 hrs 30 mins (or 1 day 6:30 hr) (Fig. 4.78.13).

Stage 14 – *Neural fold*: The embryo is further elongated and result in the appearance of a median groove in the neural plate anterior to the blastopore. The neural fold is formed at 36 hrs 45 mins (1 day 12:45 hr) and the embryo measured 1.96 ± 0.25 mm (Fig. 4.78.14).

Stage 15 – *Rotation*: The elevated neural folds growth towards each other and the neural groove became narrow. The embryo is now more elongated antero-

posteriorly. It was completed at 39 hrs 30 mins (1 day 15:30 hr) and measured 2.0 ± 0.15 mm (Fig. 4.78.15).

Stage 16 – Neural tube: The neural folds were fused completely to form the neural tube which stands out as a dorsal ridge. The embryo was further elongated and measured 2.14 ± 0.09 mm. This stage was observed at 42 hrs 30 mins or 1 day and 18:30 hr (Fig. 4.78.16).

Stage 17 – Tail bud: A small outgrowths, the tail bud protruded at the posterior end of the embryo, and became slightly curved to the left side. Bulges of gill plates are persist in the cephalic region. Completion of this stage is observed at 47 hrs 45 mins (or 1 day 23:45 hr). Embryo measured 2.29 ± 0.23 mm (Fig. 4.78.17).

Stage 18 – Muscular response: The tail buds becomes longer than wide and the embryo now has begun to loss its spherical form, so that the convexly curved line of the back becomes first straight and then gradually concave at 2 days and 2 hr (50 hrs). The embryo measured 3.50 ± 0.62 mm (Fig. 4.78.18).

Stage 19 – Heart beat: It is indicated by the pulsation of heart. Rudimentary gill buds start their appearance. Tail continued elongation. Heart beat is observed at 2 days plus 6 hr (or 54 hrs) and the embryo measured 4.31 ± 0.29 mm (Fig. 4.78.19).

Stage 20 – Gill circulation: Gills are well developed and branched into gill filaments. Opercular fold covered the base of the gills, and oral sucker became well developed. The myotomes increase in number and extend upto the tip of tail. The embryo measured 4.4 ± 0.37 mm and it is observed within 3 days and 3 hr (75 hrs) (Fig. 4.78.20).

Stage 21 – Cornea transparent: After 3 days and 10 hrs (80:45 hrs) the cornea was transparent. Tail became straight and elongated Oral suckers and nasal pits became prominent. The embryo measured 5.43 ± 0.19 mm (Fig. 4.78.21).

Stage 22 – Tail fins transparent: Tail fins become transparent, and the epidermis is pigmented. The head and trunk are distinctly demarcated. The oral suckers are prominent and nipple shaped. This stage is observed after 3 days and 21 hrs (93 hrs 15 mins). It measured 5.68 ± 0.05 mm (Fig. 4.78.22).

Stage 23 – Operculum covers gill base: After 4 days the operculum developed, and gills length shortened. The larva hatched out from the gelatin covers and it measured 5.99 ± 0.47 mm (Fig. 4.78.23).

Stage 24 – Operculum closes on right side: It is observed that right operculum fold closed after 6 days, and left external gill shortened. The larva is about 9.3 ± 0.82 mm. The body length is 3.69 ± 0.13 mm (Fig. 4.78.24).

Stage 25 – Spiracle form on left side: After 8 days, left operculum fold is closed and completion of spiracle takes place on the left side. The external gills have completely regressed and the tadpole measured 16.38 ± 8.28 mm while the body length is 7.23 ± 4.93 mm (Fig. 4.78.25).

Stage 26 – Hind limb bud < ½ its diameter: The tadpole measured 22.69 ± 1.13 mm while the body length is 11.82 ± 0.5 mm. Hind limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 15 days. Coiled intestine became visible. The length of the limb bud was wider than long. The limb bud is about 0.11 ± 0.01 mm. Melanophores are observed to be dispersed on the dorsal and ventral side. Rows of teeth were observed in both the upper and lower labium (Fig. 4.78.26).

Stage 27 – Length of limb bud $\geq \frac{1}{2}$ its diameter: The length of the limb bud length equaled to half of its diameter is observed after 19 days. The body length is 12.64 ± 0.49 mm and the total length is 23.26 ± 0.96 mm. Length of limb was 0.32 ± 0.06 mm. Dispersion of melanophores was found to be comparatively denser than in the earlier stage. Oral structure does not change (Fig. 4.78.27).

Stage 28 – Length of limb bud \geq its diameter: The body length was 13.15 ± 0.07 ; total length was 25 ± 1.13 mm; limb length was 0.65 ± 0.07 mm. Length of limb bud equaled to its diameter on the 22nd day. Similarly, in this stage melanophores were found to be denser in distribution, and the oral structure was found to be the same as in the earlier stages (Fig. 4.78.28).

Stage 29 – Length of limb bud $\geq 1 \frac{1}{2}$ its diameter: After about 25 days, the limb bud becomes more elongated and it was (0.8 ± 0.14 mm) about one and half of its width. The tadpole length was 29 ± 3.98 mm and the body length was 13.15 ± 0.07 mm (Fig. 4.78.29).

Stage 30 – Length of limb bud = twice its diameter: The limb bud further increased in length and becomes conical. The limb length was about 1.6 ± 0.14 mm (twice its width), and the tadpole length was 30.17 ± 2.69 mm and the body length was 12.6 ± 0.85 mm. The bud was still without any pigment, and there was no change in the oral structure. It is observed after 28 days (Fig. 4.78.30a&b).

Stage 31 – Foot paddle: After 30 days, melanophores were also found to be present on the base of the limb bud which had taken the shape of a spatula. The total length of the tadpole was 31.07 ± 1.41 mm from snout to tail tip. The body length was 12.7 ± 0.61 mm; the limb length was 1.63 ± 0.25 mm (Fig. 4.78.31).

Stage 32 – First interdigital indentation: The margin of the foot paddle became slightly indented on the dorsal side, which separates the prominences of the 4th and 5th toes. After 32 days the tadpole head length was 13 ± 2.83 mm and the total length was 31.75 ± 3.18 mm. At this stage, the limb bud was about 1.75 ± 0.35 mm in length (Fig. 4.78.32).

Stage 33 – Second interdigital indentation: After 34 days, the margin of the foot paddle became indented on the margin between toes 5-4 and 4-3 separating the prominence of 3rd, 4th and 5th toes. The thigh, shank and ankle with foot segment were well demarcated each other. The tadpole length was 36.35 ± 0.21 mm and the body length was 13.75 ± 2.83 mm. The limb bud length was 18 ± 0.28 mm (Fig. 4.78.33).

Stage 34 – Third interdigital indentation: The margin of foot paddle became indented on the margin between toes 5-4, 4-3 and 3-2 separating the prominence of 2nd, 3rd, 4th and 5th toes. After 36 days, the tadpole attained 37.6 ± 0.99 mm. and the head length was 18.7 ± 0.14 mm. Melanophores made a faint discontinuous streak from the distal region of thigh up to tip of toes (Fig. 4.78.34a&b).

Stage 35 – Fourth interdigital indentation: After 37 days the the total length of tadpole was about 40.27 ± 1.42 mm; the body length was 18.23 ± 0.87 mm; the hindlimb length was 2.47 ± 0.42 mm. The margin of the foot paddle was also indented between toes 1st and 2nd and all five toes were separated from each other. Melanophores were found to be more concentrated at the base of the limb, and to be dispersed towards the toe formation region (Fig. 4.78.35).

Stage 36 – Separation of 5-4 and 4-3 toes: After 39 days, the total length of tadpole was about 47.83 ± 2.75 mm; the body length was 21.98 ± 0.17 mm; the hind limb

length was 4.45 ± 0.64 mm. The 1st and 2nd toes were still joined, while the 3rd, 4th and 5th toes were separated (Fig. 4.78.36).

Stage 37 – Toes separation completed: All toes were completely separated and webbed after 41 days. The total length of tadpole was about 47.4 ± 0.14 mm; the body length was 23.75 ± 0.35 mm; the hind limb length was 5.15 ± 0.07 mm (Fig. 4.78.37).

Stage 38 – Appearance of Metatarsal tubercles: Metatarsal tubercles showed its appearance at the base of 1st toe after 43 days, and the total length of tadpole was about 58.25 ± 4.59 mm; the body length was 26.35 ± 1.2 mm; the hind limb length was 10.1 ± 5.79 mm (Fig. 4.78.38).

Stage 39 – Appearance of Subarticular patches: Subarticular tubercles first appeared as parches after 45 days, and the total length of tadpole was about 57.9 ± 4.46 mm; the body length was 26.58 ± 1.91 mm; the hind limb length was 10.65 ± 3.42 mm (Fig. 4.78.39).

Stage 40 – Completion of foot tubercles: After 47 days, toes were with fully developed subarticular patches and fully webbed. The total length of tadpole was about 64.6 ± 6.64 mm; the body length was 29.05 ± 1.84 mm; the hind limb length was 25.38 ± 3.71 mm (Fig. 4.78.40a&b).

Stage 41 – Vent tube gone: After 51 days, the more drastic changes of metamorphosis began. The cloacal tail piece was atrophied and the total of tadpole was about 64.5 ± 4.95 mm; the body length was 29 ± 1.41 mm; the hind limb length was 27.75 ± 2.47 mm (Fig. 4.78.41).

Stage 42 – Emergence of fore limbs: On the 59th day, the total length of tadpole was about 60 ± 2.83 mm; now the hind limb length was 30.05 ± 0.64 mm which is

longer than that of the body length i.e. 25.8 ± 0.57 mm. Tadpole developed both fore limbs and hind limbs. Mouth started widening, and it was anterior to nostril. Length of fore limbs measured 10 mm (Fig. 4.78.42).

Stage 43 – Mouth between nostril and eye: Resorption of tail started to regress after 61 days and the length of tadpole was about 53.95 ± 0.07 mm; the body length was 24.5 ± 0.42 mm; the hind limb length was 33.1 ± 0.14 mm. The lateral margin of mouth reached between nostril and eye. Shedding of teeth was completed (Fig. 4.78.43).

Stage 44 – Mouth beneath eye: There was further widening of mouth after 62 days and the tail was greatly reduced and measured 14.03 ± 1.26 mm. The total length of tadpole was about 37.40 ± 2.46 mm; the body length was 23.37 ± 0.57 mm; the hind limb length was 39.17 ± 0.29 mm. Formation of the tongue took place (Fig. 4.78.44).

Stage 45 – Mouth posterior to eye: After 63 days, resorption of the tail was completed and only a stub remained, which measured 2 mm, while the tadpole measured 32.40 ± 1.6 mm in length. And the mandible extended beyond the eye. Tongue had fully developed (Fig. 4.78.45).

Stage 46 – Metamorphosis completed: After 64 – 65 days, the tail was completely resorbed and the frog-let looks like an adult and measured 20.24 ± 1.8 mm (Fig. 4.78.46).

2. Developmental Stages and Metamorphosis of *Hylarana nicobarensis*: The developmental stages of *Hylarana nicobarensis* were recorded from the time of egg laying till the completion of metamorphosis at the study sites II (with water temperature 12°C - 23.5°C ; pH= 6.3 - 6.8) and IV (water temperature 12°C -

24.5°C; pH= 6.4 - 7.4) under natural environment and in the laboratory at the temperature between 13°C and 25°C. In the laboratory also, the pattern and duration of development and metamorphosis was observed to be more or less the same during the breeding season (Table 29). The development and metamorphosis was therefore completed within about 73 – 75 days in *Hylarana nicobariensis* at temperature between 13°C and 25°C in the laboratory condition. A brief account of all the 46 stages of development and metamorphosis of *Hylarana nicobarensis* is given in the following sections.

Stage 1 – *Unfertilized egg*: The freshly laid egg is spherical in shape with the animal hemisphere pigmented dark red-brown and the yolk vegetal hemisphere pale yellowish white. The animal hemisphere occupies more than half of the egg. It measures about 1.59 ± 0.01 mm in diameter. It is surrounded by a thin, transparent, vitelline membrane (Fig.4.79.1).

Stage 2 – *Fertilized egg*: A faintly gray crescent is noticeable on the animal pole of the egg. The vegetal hemisphere is more prominent than earlier. It measures about 1.75 ± 0.02 mm. This stage is observed within 35 mins (Fig.4.79.2).

Stage 3 – *Two cell stage*: After fertilization, within 1: 40 hrs a furrow extends from the animal pole down through the vegetal hemisphere. Two blastomeres of equal sizes are formed by holoblastic cleavage. Two cell stage measures about 1.64 ± 0.01 mm (Fig.4.79.3).

Stage 4 – *Four cell stage*: The second cleavage is also meridional holoblastic right angle to the first cleavage, producing four blastomeres of equal sizes. Four cell stage is observed at 2 hrs 45 mins after fertilization which measures 1.75 ± 0.02 mm (Fig.4.79.4).

Stage 5 – Eight cell stage: The third cleavage is latitudinal, near the equator of the egg producing four pigmented micromeres above and four unpigmented macromeres below. The eight cell stage is formed within 4 hrs 25 mins. The size remains more or less constant i.e. 1.76 ± 0.03 mm (Fig.4.79.5).

Stage 6 – Sixteen cell stage: The fourth cleavage furrows are meridional. Sixteen blastomeres are formed by division of each blastomere. The egg size slightly increased and measured 1.78 ± 0.01 mm which is observed after 6 hrs 25 mins (Fig.4.79.6).

Stage 7 – Thirty two cell stage: The fifth cleavage is latitudinal which is more or less delayed in vegetal hemisphere. Thirty cell stage is observed at 9 hrs and 20 mins and it measures 1.78 ± 0.08 mm (Fig.4.79.7).

Stage 8 – Mid cleavage: Division of blastomeres continuing resulting each blastomere in to smaller blastomeres. The cells in the animal pole become slightly smaller than that of vegetal hemisphere. This stage is also known as morula, and measures about 1.87 ± 0.01 mm in diameter. This stage is the beginning of blastulation or early blastula. It took place after 13 hrs 45 mins (Fig.4.79.8).

Stage 9 – Late cleavage: After 19 hrs 10 mins, the embryo is considered as blastula, and measures 2.06 ± 0.04 mm. Micromeres and macromeres are minute that makes surface smooth (Fig.4.79.9).

Stage 10 – Dorsal lip: It is the onset of invagination where the blastoporal lip extending laterally, forming a crescent shaped lateral lips after one day and the embryo measured 2.12 ± 0.02 mm (Fig.4.79.10).

Stage 11 – Mid gastrula: Lateral tips of blastoporal lip fused ventrally making blastopore circular and the animal hemisphere continuing to flatten. This stage is

also known as yolk plug stage and it took about one day and 6 hrs. The embryo measured 2.0 ± 0.06 mm (Fig.4.79.11).

Stage 12 – Late gastrula: Yolk plug becoming smaller due to invagination of the animal hemisphere. The late gastrula measured 2.06 ± 0.01 mm. It is observed after one day and 12 hrs (Fig.4.79.12).

Stage 13 – Neural plate: Yolk plug invisible and primary neural fold distinct. The embryo is slightly elongated and measured 2.12 ± 0.01 mm. This stage took about one day and 18 hrs (Fig.4.79.13).

Stage 14 – Neural fold: Secondary neural fold and neural groove distinct and the embryo is further elongated and that measured 2.30 ± 0.01 mm. The neural fold is formed after 2 days (Fig.4.79.14).

Stage 15 – Rotation: The embryo was further elongated and measured 2.31 ± 0.01 mm. This stage was observed after 2 days and 8 hrs. The elevated neural folds contacted each other dorsomedially in the whole trunk (Fig.4.79.15).

Stage 16 – Neural tube: The neural tube completed within 2 days and 13 hrs. The neural tube stands out as a dorsal ridge and the embryo is now more elongated antero-posteriorly and measured about 2.48 ± 0.01 mm (Fig.4.79.16).

Stage 17 – Tail bud: A small outgrowths, the tail bud elongated at the posterior end of the embryo; stomodium and somatic in trunk musculature distinct and dark pigmentation at position of future nares is distinct. This stage is completed within 2 days and 18 hrs. Embryo measured 2.51 ± 0.01 mm (Fig.4.79.17).

Stage 18 – Muscular response: Both dorsal and ventral portions of tail fin slightly developing and gradually distinct. Oral pit is developing and the embryo measured

2.74 ± 0.21 mm. Muscular response to mechanical stimulation is observed after 3 days and 12 hrs (Fig.4.79.18).

Stage 19 – Heart beat: It is indicated by the appearance of external gill bud and pulsation of heart. Tail continued elongation. Heart beat stage is observed after 4 days and 6 hrs and the embryo measured 3.78 ± 0.01 mm (Fig.4.79.19).

Stage 20 – Gill circulation: Tail continued elongation and gills are well developed with branching filaments. Oral sucker became well developed and the embryo measured 5.76 ± 0.11 mm and it is observed at 5 days and 4 hrs (Fig.4.79.20).

Stage 21 – Cornea transparent: Within 6 days and 8 hrs, the cornea was transparent and the embryo starts to hatch out from the gelatinous cover. Elongation of tail continued and nasal pits became prominent. The embryo measured 6.25 ± 0.03 mm (Fig.4.79.21).

Stage 22 – Tail fins transparent: The larva start to swim and the head and trunk are distinctly demarcated. Tail fins become transparent, and pigmentations on the epidermis are noticeable. This stage is observed after 7 days and it measured 8.0 ± 0.01 mm (Fig.4.79.22).

Stage 23 – Operculum covers gill base: After 10 days the operculum developed, and gills on both the sides of right and left still persists. The larva measured 8.23 ± 0.12 mm (Fig.4.79.23).

Stage 24 – Operculum closes on right side: Operculum fold on the right side is closed and only left external gill can be seen. The larva is about 8.59 ± 0.04 mm and it is observed on the 14th day (Fig.4.79.24).

Stage 25 – Spiracle form on left side: After 19 days, left operculum fold is also closed and spiracle is observed on the left side. Coiled intestine became visible and the tadpole measured 13.35 ± 0.99 mm (Fig.4.79.25).

Stage 26 – Hind limb bud $< \frac{1}{2}$ its diameter: The tadpole measured 19.72 ± 0.32 mm after 22 days and hind limb buds appeared at the junction of the trunk and tail on either sides of the cloacal tail. The length of the limb bud was wider than long. Melanophores are observed to be dispersed throughout the body and tail. Teeth rows were observed in both the upper and lower labium (Fig.4.79.26a).

Stage 27 – Length of limb bud $\geq \frac{1}{2}$ its diameter: After 25 days, the length of the limb bud equaled to half of its diameter and the total length of larva is 22.34 ± 0.52 mm. Dispersion of melanophores was continued and the oral structure does not change (Fig.4.79.26b).

Stage 28 – Length of limb bud \geq its diameter: Length of limb bud equaled to its diameter is observed on the 28th day and the total length is 24.39 ± 0.08 mm. The oral structure is same as in the earlier stages and melanophores are more denser (Fig.4.79.28).

Stage 29 – Length of limb bud $\geq 1 \frac{1}{2}$ its diameter: In this stage, the limb bud becomes more elongated and it is about one and half of its width. This stage is observed after 31 days and the tadpole length is 24.97 ± 0.18 mm (Fig.4.79.29).

Stage 30 – Length of limb bud = twice its diameter: At this stage the limb bud further increased in length and becomes conical. The length of limb bud is twice its width, and the tadpole length was 27.14 ± 0.16 mm. Pigmentation on the bud is observed and there was no change in the oral structure. It is observed after 34 days (Fig.4.79.30).

Stage 31 – Foot paddle: After 36 days, melanophores were also found to be present on the base of the limb bud which looks like the shape of a spatula. The total length of the tadpole was 27.86 ± 0.25 mm (Fig.4.79.31).

Stage 32 – First interdigital indentation: After 38 days the total length was 28.19 ± 0.20 mm. The margin of the foot paddle became slightly indented on the dorsal side, demarcates the 4th and 5th toes (Fig.4.79.32).

Stage 33 – Second interdigital indentation: After 40 days, the tadpole length was 28.79 ± 0.09 mm and the margin of the foot paddle became indented on the margin between toes 5-4 and 4-3 separating the prominence of 3rd, 4th and 5th toes. The thigh, shank and ankle with foot segment start to demarcate (Fig.4.79.33a&b).

Stage 34 – Third interdigital indentation: After 42 days, the tadpole attained 30.56 ± 0.09 mm. Melanophores are concentrated along the region of the thigh up to tip of toes. The margin of foot paddle became indented on the margin between toes 5-4, 4-3 and 3-2 separating the prominence of 2nd, 3rd, 4th and 5th toes (Fig.4.79.34).

Stage 35 – Fourth interdigital indentation: After 44 days, the total length of tadpole was about 32.48 ± 0.17 mm;. The margin of the foot paddle was also indented between toes 1st and 2nd and all five toes were separated from each other. Melanophores were found to be more concentrated at the base of the limb, and to be dispersed towards the toe formation region (Fig.4.79.35).

Stage 36 – Separation of 5-4 and 4-3 toes: After 46 days, the total length of tadpole was about 32.51 ± 0.21 mm. The 1st and 2nd toes were still joined, while the 3rd, 4th and 5th toes were separated (Fig.4.79.36).

Stage 37 – Toes separation completed: All toes were completely separated and webbed after 49 days. Pigmentation was observed up to the digits. The total length of tadpole was about 34.33 ± 0.05 mm (Fig.4.79.37a&b).

Stage 38 –Appearance of Metatarsal tubercles: The total length of tadpole was about 35.70 ± 0.13 mm. Metatarsal tubercles developed at the base of 1st toe after 52 days (Fig.4.79.38).

Stage 39 – Appearance of Subarticular patches: Patches of subarticular tubercles appeared on the ventral side of digits and the total length of tadpole was about 38.59 ± 0.08 mm. This stage was observed after 56 days (Fig.4.79.39a&b).

Stage 40 – Completion of foot tubercles: After 61 days, toes were with fully developed subarticular patches and fully webbed. The total length of tadpole was about 40.89 ± 0.15 mm (Fig.4.79.40).

Stage 41 –Vent tube gone: After 65 days, the cloacal tail piece was atrophied and the total length of tadpole was about 34.14 ± 0.15 mm (Fig.4.79.41).

Stage 42 – Emergence of fore limbs: Mouth anterior to nostril started widening, and the fore limbs start to emerge out. Normally, left limb emerges prior to right limb. This stage was observed after 69 days and the total length of tadpole was about 33.38 ± 0.20 mm (Fig.4.79.42).

Stage 43 – Mouth between nostril and eye: After 71 days, resorption of tail started and the length of tadpole was about 26.12 ± 0.21 mm. The lateral margin of mouth reached between nostril and eye. Shedding of teeth was completed (Fig.4.79.43).

Stage 44 – Mouth beneath eye: The tail was greatly reduced and the total length of tadpole was about 23.68 ± 0.14 mm There was further widening of mouth after 72 days (Fig.4.79.44).

Stage 45 – Mouth posterior to eye: After 73 days, resorption of the tail was completed and only a stub remained, and the tadpole measured 32 mm in length. And the mandible extended beyond the eye. Tongue was fully developed (Fig.4.79.45).

Stage 46 – Metamorphosis completed: After 74 – 75 days, the tail was completely resorbed and the frog-let looks like an adult and measured 20 mm (Fig.4.79.46a&b).

3. Developmental Stages and Metamorphosis of *Kaloula pulchra*: Since it was failed to do captive breeding in *Kaloula pulchra*, in order to study the embryonic development in the laboratory condition, freshly spawned eggs masses were collected from the field and observed in the laboratory with water temperature ranged from 16°C – 28°C (Table 30). The time of egg laying till the completion of metamorphosis was also monitored in the natural environment at the study sites III (water temperature 15°C – 28°C; pH= 6.0 - 6.7) and V (water temperature 14.5°C – 29°C; pH= 6.1 - 6.7). The life cycle of this species lasted for a very short period which is only about 47 days. A brief account of various stages of development and metamorphosis of *Kaloula pulchra* is given in the following.

Stage 1 – Unfertilized egg: The newly laid egg is spherical in shape with the animal pole pigmented dark brown which slowly fainted towards the creamy vegetal hemisphere. It measures about 1.48 ± 0.43 mm in diameter. It is enveloped by a thin, transparent, vitelline membrane. Around the egg is the jelly capsule (Fig.4.80.1).

Stage 2 – Fertilized egg: Within 25 minutes, a milky pigmented zone, the gray crescent starts to appear on the animal pole which indicates the penetration of male

sperm inside the female egg. It measures about 1.48 ± 0.71 mm. This stage is observed within 25 mins (Fig.4.80.2).

Stage 3 – Two cell stage: A wide furrow appears in the animal hemisphere after fertilization which extends down through the gray crescent, continued towards the vegetal hemisphere, dividing the egg into two blastomeres. The embryo measures about 1.51 ± 0.64 mm. The first cleavage was completed in 45 mins after fertilization (Fig.4.80.3).

Stage 4 – Four cell stage: The second cleavage was meridional and right angle to the first cleavage, and it started from the animal. The complete four cell stage with four blastomeres was observed at 1 hrs 05 mins after fertilization. The embryo measures 1.52 ± 0.38 mm (Fig.4.80.4).

Stage 5 – Eight cell stage: The third cleavage is horizontal, latitudinal towards the animal pole and at right angle to the earlier cleavages. Eight blastomeres have formed. The micromeres of the animal pole are pigmented and the macromeres of the vegetal pole are slightly pigmented at the upper region and slowly unpigment towards the lower region. It was observed at 1 hrs 55 mins. The size measured 1.50 ± 0.27 mm (Fig.4.80.5).

Stage 6 – Sixteen cell stage: The fourth cleavage furrows are found to be vertical and formed sixteen blastomeres by division of each blastomere. The egg size measured 1.51 ± 0.53 mm. It was observed after 2 hrs 50 mins (Fig.4.80.6).

Stage 7 – Thirty two cell stage: The fifth cleavage is horizontal and cut each blastomere completely resulting in the formation of sixteen smaller micromeres and sixteen larger macromeres. It was recorded at took about 5 hrs and measures 1.52 ± 0.84 mm (Fig.4.80.7).

Stage 8 – Mid cleavage: The morula stage is characterized by the division of earlier stage. Pigmentation on the animal pole became a little bit darker and divided equally and smaller in size, while the unpigmented vegetal pole macromeres divided unequally. The size was increased and measured about 1.53 ± 0.52 mm. in diameter. It indicated the beginning of blastulation and was observed after 6 hrs and 55 mins (Fig.4.80.8).

Stage 9 – Late cleavage: Blastulation was completed and the embryo looks granular. It took about 8 hrs and 20 mins and the size was increased and measured 1.55 ± 0.27 mm (Fig.4.80.9).

Stage 10 – Dorsal lip: At this stage, the dorsal lip of blastopore was observed below the equator due to invagination of the micromeres at 9 hrs and 30 mins where the embryo measured 1.55 ± 0.63 mm (Fig.4.80.10).

Stage 11 – Mid gastrula: Due to the continuous epibolic migration of micromeres over the vegetal hemisphere, the exposed area of macromeres was greatly reduced and formed the yolk plug stage. It took about 11 hrs 15 mins and the gastrula measured 1.59 ± 1.45 mm (Fig.4.80.11).

Stage 12 – Late gastrula: Due to continuous invagination of the micromeres the blastopore was gradually reduced at about 12 hrs and 45 mins and the slightly elongated late gastrula which measured 1.67 ± 1.29 mm in diameter was formed (Fig.4.80.12).

Stage 13 – Neural plate: The embryo continued to elongate and the flattened dorsal surface formed the neural plate, and the elevated lateral ridges formed neural folds. The embryo measured 1.84 ± 0.75 mm and this stage was observed at 14 hrs and 55 mins (Fig.4.80.13).

Stage 14 – Neural fold: The elongating embryo result in the formation of the neural fold at around 16 hrs and 40 mins and the it measured 2.39 ± 0.63 mm (Fig.4.80.14).

Stage 15 – Rotation: Each neural fold growth towards one another and the neural groove became very narrow. The embryo continued to elongate antero-posteriorly. It was completed at 18 hrs and 05 mins and measured 2.40 ± 1.19 mm (Fig.4.80.15).

Stage 16 – Neural tube: The embryo was further elongated and a dorsal ridge was formed due to the fusion of the two neural folds. The neural tube measured 2.47 ± 0.42 mm and it was observed at 18 hrs and 45 mins (Fig.4.80.16).

Stage 17 – Tail bud: It was characterized by the protrusion at the posterior end of the embryo which became slightly curved toward the left side. Gill buds persisted in the cephalic region and this stage was observed at 19 hrs 15 mins. It measured 3.01 ± 0.74 mm (Fig.4.80.17).

Stage 18 – Muscular response: The tail buds elongated and became longer than wide and dark pigmentation could be seen at positions of future nares. The embryo started to hatch at this stage. It was observed at 20 hrs 10 mins and the embryo measured 3.57 ± 0.92 mm (Fig.4.80.18).

Stage 19 – Heart beat: Both dorsal and ventral portions of tail fin slightly developed and this stage was indicated by the pulsating heart. Rudimentary external gills slightly protruded and tail continued elongation. Heart beat was observed at 22 hrs 15 mins and the embryo measured 3.90 ± 1.23 mm (Fig.4.80.19).

Stage 20 – Gill circulation: Gills were elongated and oral sucker became well developed. Mouth started to open and cornea becoming prominent. The larvae started to swim. The embryo measured 4.33 ± 1.62 mm and it was observed at 1 day and 5:30 hrs (Fig.4.80.20).

Stage 21 – Cornea transparent: After 1 day and 11 hrs the cornea was transparent. The external gills attained their maximum length and the developing operculum covered the basal portions. Tail became straight and elongated. Oral suckers and nasal pits became prominent. The embryo measured 5.63 ± 1.35 mm (Fig.4.80.21).

Stage 22 – Tail fins transparent: Dorsal and ventral tail fins become transparent, and the head and trunk are distinctly demarcated. The oral suckers and the cornea were prominent. This stage was observed after 1 day and 21 hrs and it measured 5.93 ± 1.02 mm (Fig.4.80.22).

Stage 23 – Operculum present: After 2 days and 12 hrs, the operculum was well developed, and gills length shortened. The larva was elongated and measured 6.37 ± 1.28 mm (Fig.4.80.23).

Stage 24 – Operculum closes on right side: After 2 days and 23 hrs, it was observed that right external gill was disappeared due to closing of the right operculum fold, and left external gill shortened. The larva was about 7.24 ± 1.83 mm in length (Fig.4.80.24).

Stage 25 – Spiracle form on left side: After 3 days and 15 hrs, left operculum fold was also closed and completion of spiracle took place on the ventro-medial position. Mouth shifting to anterior tip of body and the tadpole became transparent and measured 9.25 ± 2.46 mm (Fig.4.80.25).

Stage 26 – Hind limb bud $< \frac{1}{2}$ its diameter: The tadpole measured 14.31 ± 2.57 mm in length and hind limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 5 days 22 hrs. The length of the limb bud was wider than long. Melanophores were observed to be dispersed on the dorsal side of the

body and tail region. No keratodonts were observed on the mouth region (Fig.4.80.26a-c).

Stage 27 – Length of limb bud $\geq \frac{1}{2}$ its diameter: The length of the limb bud equaled to half of its diameter was observed after 6 days and 18 hrs. The tadpole measured 18.26 ± 1.82 mm in length. Dispersion of melanophores was found to be comparatively denser than in the earlier stage (Fig.4.80.27).

Stage 28 – Length of limb bud \geq its diameter: Length of limb bud equaled to its diameter on the 8th day and the total length was 20.49 ± 0.70 . In this stage melanophores were found to be denser in distribution, and the oral structure was found to be the same as in the earlier stages (Fig.4.80.28).

Stage 29 – Length of limb bud $\geq 1 \frac{1}{2}$ its diameter: On the 10th day, the limb bud becomes more elongated and it was about one and half of its width. The tadpole total length was 20.51 ± 1.03 mm (Fig.4.80.29).

Stage 30 – Length of limb bud = twice its diameter: The limb bud further increased in length and becomes conical. The length was about twice its width, and the tadpole length was 20.54 ± 0.97 mm. Patch of pigments was observed on the limb, and there was no change in the oral structure. It was observed after 12 days (Fig.4.80.30a-c).

Stage 31 – Foot paddle: A spatula-shaped limb bud was observed on the 14th day. Total length of the tadpole was 22.51 ± 0.76 mm (Fig.4.80.31).

Stage 32 – First interdigital indentation: First indentation on the interdigits separated the 4th and 5th toes on the 16th day. At this stage, the tadpole length was about 23.64 ± 1.56 mm (Fig.4.80.32).

Stage 33 – Second interdigital indentation: After indentation between toes 5-4, second interdigital indentation was observed between the 3rd and 4th toes. This stage was observed on the 18th day and the tadpole measured 24.66 ± 0.83 mm in length (Fig.4.80.33).

Stage 34 – Third interdigital indentation: After 19 days, the margin of foot paddle became indented between toes 5-4, 4-3 and 3-2 separating the prominence of 2nd, 3rd, 4th and 5th toes. At this stage the thigh, shank and ankle with foot segment were well demarcated each other and the tadpole attained 25.32 ± 0.82 mm in length. Melanophore pigmentations became more prominent (Fig.4.80.34a-c).

Stage 35 – Fourth interdigital indentation: Within 21 days where the total length of tadpole was about 26.49 ± 1.65 mm, indentation was seen between toes 1st and 2nd and all five toes were separated from each other. Melanophores were found to be more dispersed (Fig.4.80.35).

Stage 36 – Separation of 5-4 and 4-3 toes: After 23 days, the total length of tadpole was about 27.68 ± 0.67 mm. The 3rd, 4th and 5th toes were separated, while the 1st and 2nd toes were still joined. Melanophores were seen to be more concentrated in the distal part (Fig.4.80.36).

Stage 37 – Toes separation completed: All toes were completely separated and slightly elongated after 28 days. At this stage the average total length of tadpole was about 28.27 ± 1.29 mm (Fig.4.80.37).

Stage 38 –Appearance of Metatarsal tubercles: Metatarsal tubercles showed its appearance at the base of 1st toe after 31 days, and the total length of tadpole was about 28.56 ± 1.34 mm. Toes are free and no webs are seen in between digits (Fig.4.75.38).

Stage 39 – Appearance of Subarticular patches: Subarticular tubercles first appeared as patches on the ventral digits after 33 days, and the total length of tadpole was about 29.42 ± 1.53 mm (Fig.4.80.39).

Stage 40 – Completion of foot tubercles: After 35 days, toes were with fully developed subarticular patches with rudimentary webbed. The total length of tadpole was about 29.87 ± 1.26 mm (Fig.4.80.40).

Stage 41 – Vent tube gone: After 38 days, the vent tube disappeared and the protruded fore limb could be seen on either side of the lateral body. The total length of tadpole was about 29.64 ± 1.72 mm (Fig.4.80.41).

Stage 42 – Emergence of fore limbs: After 40 days, fore limbs emerged and the total length of tadpole was about 28.78 ± 0.88 mm (Fig.4.80.42a-c).

Stage 43 – Mouth between nostril and eye: Resorption of tail started to regress after 43 days and the length of tadpole was about 23.28 ± 1.74 mm. The lateral margin of mouth reached between nostril and eye (Fig.4.80.43).

Stage 44 – Mouth beneath eye: There was further widening of mouth after 45 days and the tail was greatly reduced and measured 15.53 ± 2.04 mm. Formation of the tongue took place (Fig.4.80.44).

Stage 45 – Mouth posterior to eye: After 46 days, resorption of the tail was completed and only a stub remained, while the tadpole measured 12.16 ± 0.64 mm in length. And the mandible extended beyond the eye. Tongue had fully developed (Fig.4.80.45).

Stage 46 – Metamorphosis completed: After 46 days, the tail was completely resorbed and the frog-let looks like an adult and measured 9.07 ± 0.47 mm (Fig.4.80.46a&b).

The development and metamorphosis was therefore completed within about 47 days in *Kaloula pulchra* at temperature between 16° C and 28° C in the natural environment. In the laboratory also, the pattern and duration of development and metamorphosis was observed to be more or less the same during the breeding season.

4. Developmental Stages and Metamorphosis of *Microhyla berdmorei*: As in the case of *Kaloula pulchra*, breeding in captivity was failed and the freshly spawned eggs masses were collected from the field and the embryonic development was studied in the laboratory where water temperature ranged between 13 °C – 24 °C (Table 31). The completion of metamorphosis from the newly laid egg was also observed in the natural environment at the study sites II (water temperature 12 °C - 23.5 °C; pH= 6.3 - 7.1) and IV (water temperature 12 °C - 24.5 °C; pH= 6.4 - 7.4). The duration of metamorphosis from the time of egg laying is about 109 days. A brief account of complete 46 stages of development and metamorphosis of *Microhyla berdmorei* is given in the following.

Stage 1 – Unfertilized egg: The freshly layed egg is spherical in shape with the animal hemisphere pigmented yellowish to light brown and the yolk vegetal hemisphere almost creamy white. It measures about 1.43 ± 0.02 mm in diameter. It is surrounded by a thin, transparent, vitelline membrane. Around the egg is the jelly capsule which swells on contact with water (Fig.4.81.1).

Stage 2 – Fertilized egg: A pigmented zone, the gray crescent is visible on dorsal side of the egg. It measures about 1.43 ± 0.04 mm. This stage is observed within 25 mins (Fig.4.81.2).

Stage 3 – Two cell stage: After fertilization, a furrow extends from the animal pole down through the vegetal hemisphere, dividing the egg into two blastomeres. The cleavage furrow passed through the gray crescent measuring about 1.45 ± 0.03 mm. The first cleavage was completed in 1 hr 05 mins after fertilization (Fig.4.81.3).

Stage 4 – Four cell stage: The second cleavage was meridional plane at right angle to the first cleavage, and progressed from the animal pole and dividing the egg into four blastomeres. The complete four cell stage was observed at 2 hrs 40 mins after fertilization. The embryo measures 1.45 ± 0.15 mm (Fig.4.81.4).

Stage 5 – Eight cell stage: The embryo increases in size i.e. 1.47 ± 0.04 mm. The third cleavage is horizontal, slightly above the equator of the egg and at right angle to both the first and second cleavages. Out of the eight blastomeres, the four smaller micromeres of the animal pole are pigmented and the four bigger macromeres of the vegetal pole are unpigmented. Completion of the eight cell stage was recorded at 4 hrs 15 mins (Fig.4.81.5).

Stage 6 – Sixteen cell stage: Sixteen blastomeres are formed by vertical division of each blastomere. The egg does not increase in size and measured 1.47 ± 0.02 mm. It is observed after 5 hrs 25 mins (Fig.4.81.6).

Stage 7 – Thirty two cell stage: The fifth cleavage which is horizontal and asynchronous results in the formation of a rough granulated embryo. The cleavage furrow cuts the micromere completely and equally but the furrow in the vegetal hemisphere divides the macromeres unequally resulting in the formation of sixteen smaller micromeres and sixteen larger macromeres. This stage took 7 hrs and 10 mins to complete and measures 1.47 ± 0.17 mm (Fig.4.81.7).

Stage 8 – Mid cleavage: The blastomeres i.e. micromeres and macromeres continued to divide. The cells in the animal pole become smaller while the cells of the vegetal hemisphere remain larger. This morula stage measures about 1.47 ± 0.02 mm in diameter. This stage indicates the beginning of blastulation. The mid cleavage stage took place after 8 hrs 55 mins (Fig.4.81.8).

Stage 9 – Late cleavage: Due to repeated divisions of the blastomeres, the micromeres are very minute in size. This blastula stage measures 1.49 ± 0.03 mm and it is observed at 10 hrs 10 mins (Fig.4.81.9).

Stage 10 – Dorsal lip: This embryonic stage indicated the beginning of gastrulation. Due to invagination of the micromeres, a crescent shaped i.e. the dorsal lip of blastopore appeared slightly below the equator on the dorsal side of the embryo at 11 hrs 10 mins. The embryo measured 1.66 ± 0.04 mm (Fig.4.81.10).

Stage 11 – Mid gastrula: Yolk plug is formed due to continuation of epibolic migration of micromeres over the vegetal hemisphere. It took about 12 hrs 10 mins and the gastrula measured 1.84 ± 0.05 mm (Fig.4.81.11).

Stage 12 – Late gastrula: Due to further constriction of the blastoporal lips, the small protruding blastopore gradually is reduced. The late gastrula measured 1.75 ± 0.05 mm and the whole process takes about 13 hrs 40 mins (Fig.4.81.12).

Stage 13 – Neural plate: The embryo is slightly elongated. The dorsal surface has flattened to form the neural plate while the lateral ridges became slightly elevated forming neural folds. The embryo measured 1.88 ± 0.04 mm. This stage is observed at 16 hrs 40 mins (Fig.4.81.13).

Stage 14 – Neural fold: A median groove is formed in the neural plate anterior to the blastopore. The embryo is further elongated and this stage is observed at 18 hrs 40 mins and the embryo measured 1.96 ± 0.07 mm (Fig.4.81.14).

Stage 15 – Rotation: The embryo is now more elongated antero-posteriorly. The neural groove became narrow due to the elevation of neural folds towards each other. It was completed at 20 hrs 40 mins and measured 2.23 ± 0.04 mm (Fig.4.81.15).

Stage 16 – Neural tube: The neural tube which stands out as a dorsal ridge was formed by the fusion of the neural folds. The embryo was further elongated and measured 2.35 ± 0.03 mm. This stage was observed at 22 hrs 40 mins (Fig.4.81.16).

Stage 17 – Tail bud: A protruded tail bud was observed at the posterior end of the embryo as a small outgrowth and became slightly curved to the left side. Bulges of gill plates are persist in the cephalic region. Tail bud stage was observed at 24 hrs 10 mins. Embryo measured 2.46 ± 0.03 mm (Fig.4.81.17).

Stage 18 – Muscular response: The tail bud becomes elongated, both dorsal and ventral portions of tail fin developed. Dark pigmentation was observed at positions of future nares. It was observed at 1 day and 1 hr 40 mins. The embryo measured 2.66 ± 0.03 mm (Fig.4.81.18).

Stage 19 – Heart beat: Tail continued elongation and it is indicated by the pulsation of heart. Rudimentary gill buds start their appearance. The embryo begins to hatch and heart beat was observed at 1 day and 3:40 hr and the embryo measured 3.19 ± 0.14 mm (Fig.4.81.19).

Stage 20 – Gill circulation: Gills are well developed and covered by opercular fold at their base. Oral sucker became well developed. Future nares slightly concave.

The embryo measured 4.09 ± 0.18 mm and it was observed at 1 day 5:40 hr (Fig.4.81.20).

Stage 21 – Cornea transparent: After 1 day and 12:40 hr the cornea was transparent. Heart beat and blood circulation visible from outside. Tail became straight and elongated and the embryo measured 5.83 ± 0.08 mm (Fig.4.81.21).

Stage 22 – Tail fins transparent: This stage is characterised by a distinctly demarcation between head and trunk. Tail fins become transparent, and the epidermis is pigmented. The oral suckers are prominent and this stage is observed after 2 days and 12 hr. It measured 5.88 ± 0.04 mm (Fig.4.81.22).

Stage 23 – Operculum present: After 3 days and 6 hrs, epidermis started to be transparent. The operculum was well developed, and gills length shortened. The larva was elongated and measured 6.3 ± 0.11 mm (Fig.4.81.23).

Stage 24 – Operculum closes on right side: After 5 days and 4 hrs, it was observed that right operculum fold closed and left external gill shortened. The larva was about 6.71 ± 0.05 mm (Fig.4.81.24).

Stage 25 – Spiracle opening at ventro-medial position: After 8 days, left operculum fold was closed and completion of spiracle took place at ventro-medial position. The external gills have completely regressed and the body became transparent and the tadpole measured 10.84 ± 0.41 mm (Fig.4.81.25a&b).

Stage 26 – Hind limb bud $< \frac{1}{2}$ its diameter: The tadpole measured 17.06 ± 1.14 mm. Hind limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 17 days. Coiled intestine became visible. The length of the limb bud was wider than long. Melanophores are observed to be dispersed on the dorsal and ventral side. No denticles were observed in the mouth cavity (Fig.4.81.26).

Stage 27 – Length of limb bud $\geq \frac{1}{2}$ its diameter: The length of the limb bud equal to half of its diameter was observed after 24 days. The total length of larva was 17.71 ± 1.1 mm. No denticles were observed in the mouth cavity (Fig.4.81.27).

Stage 28 – Length of limb bud \geq its diameter: Length of limb bud equal to its diameter within 42 days. The total length of larva was 18.4 ± 1.08 mm. Melanophores were found to be denser in distribution, and the oral structure was found to be the same as in the earlier stages (Fig.4.81.28).

Stage 29 – Length of limb bud $\geq 1 \frac{1}{2}$ its diameter: After about 47 days, the tadpole total length was 18.85 ± 0.62 mm and the limb bud becomes more elongated and it was about one and half of its width (Fig.4..219).

Stage 30 – Length of limb bud = twice its diameter: The limb bud further increased in length and becomes conical with pigmented on the apical region. The length was twice its width, and the tadpole length was 20.97 ± 0.33 mm. It was observed after 52 days (Fig.4.81.30a&b).

Stage 31 – Foot paddle: At this stage, terminal portion of hind limb became an oar-shaped. The total length of the tadpole was 21.44 ± 1.19 mm from snout to tail tip. This stage was observed within 56 days (Fig.4.81.31).

Stage 32 – First interdigital indentation: After 59 days, shallow indentation appeared in the margin of foot paddle between 4th and 5th toes. At this stage, the tadpole total length was 21.44 ± 1.37 mm (Fig.4.81.32).

Stage 33 – Second interdigital indentation: Shallow indentation appeared in the margin of foot paddle between 3rd and 4th toes within 61 days,. At this stage, the tadpole total length was 23.17 ± 0.29 mm (Fig.4.81.33).

Stage 34 – Third interdigital indentation: After 64 days, the tadpole attained 24.13 ± 0.04 mm. Shallow indentation appeared in the margin of foot paddle between 2nd and 3rd toes within 61 days separating the prominence of 2nd, 3rd, 4th and 5th toes (Fig.4.81.34a&b).

Stage 35 – Fourth interdigital indentation: After 67 days the total length of tadpole was about 24.31 ± 0.62 mm. The margin of the foot paddle was also indented between toes 1st and 2nd and all five toes were separated from each other. Femur, tibia and foot were distinct from each other (Fig.4.81.35).

Stage 36 – Separation of 5-4 and 4-3 toes: Within 71 days, the total length of tadpole was about 24.41 ± 0.61 mm. The 1st and 2nd toes were still joined, while the 3rd, 4th and 5th toes were separated (Fig.4.81.36).

Stage 37 – Toes separation completed: At this stage, all toes were completely separated and webbed after 74 days. The total length of tadpole was about 24.53 ± 1.77 mm (Fig.4.81.37).

Stage 38 – Appearance of Metatarsal tubercles: After 78 days, metatarsal tubercles showed its appearance at the base of 1st toe and the total length of tadpole was about 24.75 ± 2.29 mm (Fig.4.81.38a&b).

Stage 39 – Appearance of Subarticular patches: At this stage, subarticular tubercles first appeared as patches after 82 days, and the total length of tadpole was about 24.82 ± 1.94 mm (Fig.4.81.39).

Stage 40 – Completion of foot tubercles: Within 92 days, toes were with fully developed foot tubercles and fully webbed. The total length of tadpole was about 24.88 ± 1.73 mm (Fig.4.81.40).

Stage 41 –Vent tube gone: After 99 days, the cloacal tail piece was disappearing and protrusion of fore limb was observed on either side of the body. The total length of tadpole was about 24.73 ± 1.47 mm (Fig.4.81.41).

Stage 42 – Emergence of fore limbs: Emergence of fore limb was observed within 105 days. The total length of tadpole was about 22.25 ± 2.07 mm (Fig.4.81.42).

Stage 43 – Tail Atrophies: At this stage, tail was rapidly diminished, head was narrowing, mouth widening and shifted to anterior tip of head. This was observed after 106 days and the length of tadpole was about 15.41 ± 1.35 mm (Fig.4.81.43).

Stage 44 – Tail Greatly Reduced: After 107 days, eyeballs started to protrude and spiracle was disappearing. There was further widening of mouth and the tail was greatly reduced the total length of tadpole was about 13.87 ± 0.34 mm (Fig.4.81.44).

Stage 45 – Tail Stub: After 108 days, resorption of the tail was completed and only a stub remained and the tadpole measured 10.28 ± 0.07 mm in length. Tongue had fully developed (Fig.4.81.45).

Stage 46 – Metamorphosis completed: After 109 days, the tail was completely resorbed making cloaca visible from above and the frog-let looks like an adult and measured 8.21 ± 0.57 mm (Fig.4.81.46a&b).

The development and metamorphosis was therefore completed within about 109 days in *Microhyla berdmorei* at temperature between 13°C and 24°C in the natural environment. In the laboratory also, the pattern and duration of development and metamorphosis was observed to be more or less the same during the breeding season.

IV. Food and feeding behavior in relation to oral structures and intestines:

Qualitative analysis of gut contents of the tadpoles of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* revealed that the larvae started feeding from stage 25 onwards. The food items found at different stages of development of the tadpoles of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* are shown in Table 32, 33, 34 and 35; Fig. 4.87.

Larval foods of *Euphlyctis cyanophlyctis*:

Stage 25: It was observed that tadpoles start feeding from this stage onwards. The tadpoles fed mostly on detritus and phytoplanktons which include 5 classes, Bacillariophyceae: *Cymbella*, *Navicula*, *Pinnularia*, *Surirella*, *Tabellaria*, *Closterium*, Chlorophyceae: *Cosmarium*, *Docidium*, *Mougeotia*, *Oedogonium*, *Sirogonium*, *Spirogyra*, *Staurastrum*, *Pediastrum*, *Ulothrix*, Cyanophyceae: *Oscillatoria*, *Anabaena*, Cryptophyceae: *Arcella*, *Cryptomonas*, and Euglenophyceae: *Phacus* (Table 32; Fig. 4.87).

Stages 26 – 30: The gut content of the tadpoles at these stages was found to be similar with the previous stage except the additions of phytoplankton like *Diatoma* and *Suirirella* (Bacillariophyceae), *Micrasterias* (Chlorophyceae), *Anabaena*, *Aphanothece*, *Nostoc*, *Pandorina* and *Spirulina* (Cyanophyceae), *Euglena* (Euglenophyceae), and zooplankton like *Brachionus*, *Chydorus*, *Euglypha* and *Lecane* (Table 32; Fig. 4.87).

Stages 31 – 41: At these stages, apart from the food items of earlier stages, analysis of the gut content revealed that the tadpoles also feeds on phytoplankton like

Stauroneis (Bacillariophyceae), and zooplankton which includes *Diffugia* and *Paramecium* (Table 32; Fig. 4.87).

Stages 42 – 46: During this crucial period of metamorphosis, the tadpole loses its larval feeding structures and the tadpoles have stop feeding. Analysis of the gut contents shows that feeding activity have ceased. After metamorphosis the froglet starts feeding on a carnivorous diet.

Adult foods of *Euphlyctis cyanophlyctis*: The stomach content of the adult frogs was analyzed and it was found that the adults feed mostly on aquatic and terrestrial insects which include Coleoptera, Diptera (e.g. mosquito and others), Hemiptera (e.g. waterbug), Hymenoptera (e.g. winged and wingless ants), Isoptera (e.g. termites), Odonata (e.g. dragonfly), and Annelida (e.g. earthworm), pieces of leaves, twigs and plant debris (Fig. 4.107).

Oral structures of the tadpoles of *Euphlyctis cyanophlyctis*: From the present observation, it is clear that the tadpole of *Euphlyctis cyanophlyctis cyanophlyctis* is more of a bottom dweller, scraping algae and also feeding on macrophytes with the help of its heavy and keratinized beaks (Fig. 4.82&4.83).

Morphological features of the oral structure of the tadpoles of *Euphlyctis cyanophlyctis* was studied using stereoscopic binocular microscope and also with scanning electron microscope (Fig. 88a&b and Fig. 89). *Euphlyctis cyanophlyctis* tadpoles are benthic dwellers and their mouth is ventrally situated. There is no ontogenetic variation in labial teeth rows of *Euphlyctis cyanophlyctis*. The labial teeth row formula (LTRF) i.e. 1/2 is constant throughout the larval stages until the metamorphs stage.

Stage 25: Oral disc is a prominent feature of the ventral profile of the body. It is roundish, slightly broader than long, with broad anterior and posterior labia. Anterior (upper) labium (AL) is indistinct with a single continuous row of keratinized dark brown elongated teeth extending along its outer border. The margin of cup-like anterior and posterior labia on each side of the oral disc is beset with thick, rounded and elongated marginal papillae (MP) and submarginal papillae (SM). Posterior (lower) labium (PL) has two continuous rows of labial teeth and a pair of papillated palpal cups. Teeth are smooth, without indentation but scythe-shaped, and no keratinized spurs (or cusps). Therefore, the labial tooth row formula (LTRF) is 1/2. There is a single tooth row per ridge (= uniserial). The keratinized teeth are peculiar, elongated, curved, slightly pointed and embedded in the labial tissue. The jaw sheaths are wide, thick robust, dark brown, and prominent. They are provided with sharp fine serrated cutting edge. The lower jaw sheath is broad, serrated and V-shaped (Fig. 4.89).

Stage 26-30: The mouth is widening and more prominent than the previous stages and the LTRF was maintained in this stage (Fig. 4.90a). Scanning electron microscopic studies of the tadpoles of *Euphlyctis cyanophlyctis* showed the presence of both marginal papillae (MP) and submarginal papillae (SM) on each lateral side of upper and lower labium (Fig. 4.90b). At stage 28 the width of the oral structure measured 1167 μm and the length measured 1100 μm . In the upper and lower labia papillae are present only on the lateral sides. V-shaped lower jaw sheath and rows of keratodont was observed. Length of the keratodont on the anterior (upper) labium at this stage measured 73.64 - 78.9 μm , and the width at the base measured 26.3 μm at the middle region 21.04 μm and at the tip region 2.86 μm

respectively (Fig. 4.91a&b). Widths of the upper and V-shaped lower jaw sheath were 916.85 μm and 667 μm , respectively. The length of the pointed edge of the upper jaw sheath measured 52.6 μm and the width measured 36.82 μm . Magnified view of the V-shaped lower jaw sheath is serrated and pointed, the width of the opening of the V-shaped lower jaw sheath was 100.02 μm and the length measured 66.68 μm . The length of the serrated edges in the lower jaw sheath measured 31.56 μm and width 26.3 μm (Fig. 4.92a).

Stage 31-41: Labial teeth row formula was still 1/2 in these stages. The mouth is widened and the oral disc is larger than the early stages. All the rows of teeth on the upper and lower labia are well developed. Scanning electron microscopic studies of the entire oral structure of the tadpole of *Euphlyctis cyanophlyctis* at stage 37 is shown in Fig. 4.92b. The width of the oral structure measured 1737.94 μm with a length of 1199.64 μm . The upper jaw sheath measured 1153.5 μm in width with a length of 276.84 μm , while the serrated edges (suprarostrodonts) measured 28.8 μm in width and 57.69 μm in length. The serrated edges of the jaw sheath were found to be pointed throughout (Fig. 4.93a). It was also observed that the upper labium with a single keratodont row and the lower labium with two rows of keratodont. Each keratodont is slender, tapers from the middle and curve pointed at the tip. A magnified viewed of a row of keratodont in the upper labium is shown in Fig. 4.93b, and the length of a keratodonts ranged between 75.95 μm - 108.5 μm with a width of 15.19 μm , 10.85 μm and 4.34 μm at the base, middle and tip respectively. The keratodonts on the posterior labium ranged 62.92 μ - 94.38 μ in length with a width of 25.74 μ , 20.02 μ and 2.86 μ at the base, middle and tip respectively.

With the help of scanning electron microscopic studies, the oral apparatus of tadpole at stage 40 was shown in Fig. 4.94a. The width of the oral structure measured 1666.67 μm with a length of 1000.2 μm . The upper jaw sheath measured 1500.3 μm in width with a length of 250.85 μm , while the pointed serrated edges (suprarostrodonts) measured 34.19 μm in width and 47.34 μm in length. Fig. 4.89b showed a magnified viewed of a degenerating single row of keratodont in the anterior labium where the length of a keratodonts ranged between 41.25 μm – 100 μm . The width at base, middle and tip are 21.25 μm , 18.75 μm and 2.5 μm respectively. The keratodonts on the posterior labium ranged 85.71 μm - 92.95 μm in length with a width of 10.01 μm , 8.58 μm and 2.86 μm at the base, middle and tip respectively (Fig. 4.95). From stage 42 onwards, shedding of keratodonts followed by jaw sheaths takes place and the metamorphs stop feeding. Within a few days, mouth widens and resembled the adult type, which finally metamorphosed into a froglet at stage 46. From this stage, the animal starts feeding on a carnivorous diet.

Larval foods of *Hylarana nicobariensis* :

Stage 25: After the completion of spiracle formation, tadpoles started swimming and feeding. Analysis of the gut contents revealed phytoplanktons consisting of *Navicula* and *Tabellaria* (Bacillariophyceae), *Cladophora*, *Cosmarium*, *Docidium*, *Mougeotia*, *Oedogonium*, *Sirogonium*, *Spirogyra*, *Staurastrum*, *Stauroneis*, *Pediastrum* and *Ulothrix* (Chlorophyceae), *Oscillatoria* (Cyanophyceae), *Arcella* and *Cryptomonas* (Cryptophyceae), and *Phacus* (Euglenophyceae) (Table 33; Fig. 4.87).

Stages 26 – 30: Studies on the food items in these stages shows some more items in addition to stage 25. Apart from the previous stage the gut contents include phytoplankton like *Diatoma* (Bacillariophyceae), *Closterium* (Chlorophyceae), *Anabaena* and *Nostoc* (Cyanophyceae), *Euglena* and *Phacus* (Euglenophyceae), and a zooplankton, *Lecane* (Table 33; Fig. 4.87).

Stages 31 – 41: At these stages, apart from the food items of earlier stages, analysis of the gut content revealed that the tadpoles consumed some more zooplankton like *Brachionus*, *Centropyxis*, *Chydorus*, *Diffugia* and *Paramecium* (Table 33; Fig. 4.87).

Stages 42 – 46: As in the case of *Euphlyctis cyanophlyctis*, the tadpole loses its larval feeding structures and ceased feeding in these stages. After attaining stage 46, the herbivores turn into carnivores.

Adult Foods of *Hylarana nicobariensis*: The stomach content of the adults was analyzed and it was found that the adults feed mostly on insects which include Coleoptera (e.g. beetle), Dipteran flies, Hemiptera, Hymenoptera (e.g. winged and wingless ants), Isoptera (winged termites), Odonata, Orthoptera (e.g. grasshopper, acrydiidae), Annelids (e.g. earthworm), plant debris like pieces of leaves, twigs, and sand particles (Fig. 4.108).

Oral structures of the tadpoles of *Hylarana nicobariensis*: From the present investigation, the tadpole of *H. nicobariensis* is known to inhabit mostly at or near the bottom, rasping food from submerged surfaces with the help of its serrated keratinized jaw sheath.

The oral morphology of the tadpoles of *Hylarana nicobariensis* was studied with the help of stereoscopic binocular microscope and scanning electron

microscope (Fig. 4.96a&b and Fig. 4.97). As in the case of *Euphlyctis cyanophlyctis*, the labial teeth row formula (LTRF) does not change throughout the larval stages until the metamorphs stage.

Stage 25: The disc is subterminal and it bears a single row of subterminal papillae, accompanied by smaller submarginal papillae (SM) with circular bases located in the commissural region. The upper marginal papillae (MP) are interrupted medially to form a wide rostral gap (G) while the lower marginal papillae are longer and continuous throughout the labium. The jaw sheaths are wide, thick robust, and finely serrated. The upper jaw sheath (UJS) is wide and the frontal serrations slightly larger and more worn than the lateral ones. The lower jaw sheath (LJS) is slender, serrated and V-shaped (Fig. 4.97). The papillae lie on each side of the jaw sheaths (Fig. 4.98a). There are five keratodonts rows, two anteriorly (A) and three posteriorly (P). There is a single tooth row per ridge (= uniserial). Tooth rows A-1, P-2, and P-3 are complete and extend from one commissure to the other end, whereas A-2 and P-1, are interrupted medially, making the labial tooth row formula (LTRF) 2(2)/3(1). The width of the oral structure measured 1025 μm and the length measured 560 μm . Widths of the upper and V-shaped lower jaw sheath were 534.6 μm and 335.2 μm , respectively. The width of the opening of the V-shaped lower jaw sheath was 185.42 μm and the length measured 86.24 μm . The length of the serrated edges in the lower jaw sheath measured 20.25 μm and width 10.32 μm (Fig. 4.98b). Length of the keratodont on the anterior (upper) labium at this stage measured 15.74 - 22.5 μm , and the width at the base measured 10.03 μm at the middle region 8.5 μm and at the tip region 9.64 μm respectively.

Stage 26-30: Scanning electron microscopic studies of the tadpoles of *Hylarana nicobariensis* showed the presence of prominent papillae on each lateral side and lower labium. The mouth is more prominent than the previous stages and the LTRF was maintained in this stages. At stage 29 the width of the oral structure measured 1260 μm and the length measured 780 μm . Widths of the upper and V-shaped lower jaw sheath were 406.25 μm and 280.42 μm , respectively. The length of the pointed edge of the upper jaw sheath measured 25.06 μm and the width measured 20.48 μm (Fig. 4.99a). Length of the keratodont on the anterior (upper) labium at this stage measured 30.24 - 70.56 μm , and the width at the base measured 10.35 μm at the middle region 8.23 μm and at the tip region 9.06 μm respectively. As in the previous stage, the ventral papillae occur in two rows that project in different directions was seen in these stages. Each keratodont has three distinct zones, base, neck, and head. The head is convex, oblong or slightly wider in its distal extreme, and 8 to 12 cusps (Fig. 4.99b).

Stage 31-41: The mouth is widened and the oral disc is more prominent than the early stages. In these stages, the LTRF was still 2(2)/3(1) with uniserial tooth row. Scanning electron microscopic studies of the entire oral structure of the tadpole of *Hylarana nicobariensis* at stage 41 was shown in Fig. 4.100a. The width of the oral structure measured 1982.34 μm with a length of 1394.42 μm . The upper jaw sheath measured 954.65 μm in width with a length of 250.18 μm , while the serrated edges measured 16.28 μm in width and 25.64 μm in length. The length of a keratodonts ranged between 30.75 μm - 70.84 μm with a width of 10.45 μm , 8.12 μm and 9.42 μm at the base, middle and tip respectively. Each keratodont bears 8 to 12 cusps.

From stage 42 onwards, shedding of keratodonts followed by jaw sheaths and papillae takes place and the metamorphs stop feeding. The remnants of papillae could be seen in the Fig. 4.100b. Within a few days, mouth widens and modified into the adult type, which finally metamorphosed into a froglet at stage 46. From this stage onwards, the animal starts feeding on a carnivorous diet.

Larval foods of *Kaloula pulchra*:

Stage 25: Analysis of the gut contents of stage 25 revealed phytoplankton namely, *Diatoma*, *Navicula*, *Stauroneis* and *Tabellaria* (Bacillariophyceae), *Cosmarium*, *Crucigenia* and *Scenedesmus* (Chlorophyceae), *Anabaena*, *Nostoc* and *Oscillatoria* (Cyanophyceae), *Arcella* and *Cryptomonas* (Cryptophyceae) (Table 34; Fig. 4.87).

Stages 26 – 30: Though the gut contents of stage 25 and stages of hind limb bud development are very similar, apart from the previous stage, phytoplankton like *Pinnularia* (Bacillariophyceae), *Closterium* (Chlorophyceae), and *Euglena* (Euglenophyceae), and a zooplankton *Lecane* were also observed (Table 34; Fig. 4.87).

Stages 31 – 41: During toe development, in addition to the food items of earlier stages, gut content analysis revealed *Phacus* (Euglenophyceae) and a zooplankton *Paramecium* (Table 34; Fig. 4.87).

Stages 42 – 46: After the emergence of fore limb, the oral apparatus of tadpoles start to degenerate which consequence with ceased feeding. From stage 46 onwards, the animal starts to feed on other small invertebrates.

Adult foods of *Kaloula pulchra*: The stomach content of the adults was analyzed and it was found that the adults feed mostly on small insects in which Isopterans

(wingless and winged termites) shows the highest, followed by other insects Coleoptera (e.g. beetle, Curculionidae), Dipteran flies, Hymenoptera (e.g. winged and wingless ants), Orthopteran nymphs, and also include nematodes and Annelids (e.g. earthworm). Pieces of leaves and twigs are also recovered (Fig. 4.109).

Oral structure of the tadpoles of *Kaloula pulchra*: From the present study based on the light and electron micrographs, the larvae of *Kaloula pulchra* show no keratinized structures, such as jaws and labial teeth; in addition they have a terminally-oriented, as opposed to an antero-ventrally oriented mouth (umbelliform) known as semicircular labial flap (Figs 4.101a&b and Fig.4.102). They are filtering food particles throughout the water column. At stage 42, the tadpole stopped feeding and the umbelliform mouth part is degenerated and gradually transformed in to adult mouth (Fig. 4.103).

Larval foods of *Microhyla berdmorei* :

Stage 25: The gut contents of stage 25 consists of phytoplankton, *Diatoma*, *Fragilaria*, *Navicula*, *Stauroneis*, *Synedra* and *Tabellaria* (Bacillariophyceae), *Cladophora*, *Closterium*, *Cosmarium*, *Spirogyra*, *Staurastrum* and *Ulothrix* (Chlorophyceae), *Oscillatoria* (Cyanophyceae), *Arcella* and *Cryptomonas* (Cryptophyceae), and a zooplankton, *Lecane* (Table 35; Fig. 4.87).

Stages 26 – 30: Apart from the previous stage, gut content analysis of stages during hind limb bud development revealed phytoplankton like *Cymbella*, *Melosira* and *Pinnularia* (Bacillariophyceae), *Mougeotia*, *Oedogonium* and *Sirogonium* (Chlorophyceae), *Anabaena* and *Nostoc* (Cyanophyceae), and *Euglena*

(Euglenophyceae), and a zooplankton, *Euglypha* were also recovered (Table 35; Fig. 4.87).

Stages 31 – 41: Examinations on the gut contents on these stages showed *Phacus* (Euglenophyceae), and from zooplankton, *Centropyxis* and *Paramecium* as additional items apart from the rest (Table 35; Fig. 4.87).

Stages 42 – 46: After the emergence of fore limb, the oral apparatus of tadpoles start to degenerate which consequence with ceased feeding. From stage 46 onwards, the animal starts to feed on other small invertebrates.

Adult foods of *Microhyla berdmorei*: The stomach contents showed that the adults feed mostly on small insects in which Hymenopterans (especially Formicidae, e.g. ants) shows the highest followed by other insects like, Isopterans (termites), small Coleopterans, Dipterans, and also include pieces of chara, dry leaves, bamboo leaves and nematodes (Fig. 4.110).

Oral structure of the tadpoles of *Microhyla berdmorei*: The light and electron micrographs of the mouthparts of *Microhyla berdmorei* showed that they are keratinized tooth row as well as jaw sheath. They also filter particles in or near the surface film with anteriorly oriented up-turned (umbelliform) semicircular labial flap. A U-shaped medial notch is protruded out from the mouth in between the two semicircular labial flaps (Fig. 4.104 and Fig. 4.105). As the tadpoles ceased to feed from stage 42 onwards, the mouthparts gradually changed into the adult type (Fig. 4.106a&b).

Histological study on the intestines of tadpole and adult:

The present findings indicated that the intestines of tadpoles of the four species remodeled during metamorphosis. The tadpole intestine consists of two spiral coils: the outer coil (duodenum and anterior ileum) reverses direction at the switchback point and is followed by the inner coil (posterior ileum and colon), which terminates at the rectum (Fig. 4.111a). 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.

Euphlyctis cyanophlyctis: It was found that the length of the intestines at pre- and prometamorphosis is increased and abruptly decreased in the metamorphic climax (Table 36a). From stage 25 to stage 40, there is significant positive correlation between the tadpole length and the gut length at the 0.01 level (2-tailed), where $p < 0.01$ (Table 36b). At these stages, the tadpoles are feeding, and the intestine was observed to be a simple tubular structure consisting of larval epithelial cells (Fig. 4.111a). Histological study revealed that at this stage the brush border appears to be long and compact made up of primary epithelium surrounded by submucosa which is further enclosed by a thin serosa (Fig. 4.111b). The maximum length of the intestine (337.24 mm) is seen at stage 40 measuring where the tadpole reach a maximum total length, 65.86 mm. From stage 41 to stage 46, the shortening of total body length correlates with the shortening of intestines, where correlation is

significant at the 0.05 level (2-tailed; $p=0.047$) as shown in the Table 36c. Within 14 – 18 days the *Euphlyctis cyanophlyctis* tadpole intestine (from stage 40 – stage 46) shortens in length by about 85%. At stage 46 primary epithelium of tadpole intestinal tissue is already replaced by secondary epithelium. 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 (IF) appear as several circular folds that run longitudinally and are straight along the gut axis, gradually increasing in number and height, lined by mucus membrane, and finally being modified into longitudinally zigzagged folds. The zigzag folds then remain throughout adulthood (Fig. 4.111c&d). It has elaborate connective tissue and muscles.

Hylarana nicobariensis: As in the case of *Euphlyctis cyanophlyctis*, the length of intestine is increased from stage 25 to stage 40 (Table 37a), there was significant positive correlation between the tadpole length and the gut length, where $p<0.01$ at the 0.01 level (2-tailed) as shown in the Table 37b. Histological study revealed that the primary epithelia are surrounded by submucosa which is further enclosed by a thin serosa (Fig. 4.112a&b). The maximum length of the intestine (74.56 mm) is seen at stage 40 measuring where the tadpole reached a maximum total length, 40.94 mm. From stage 40 to stage 46, the shortening of total body length correlates with the shortening of intestines, where $p<0.01$ at the 0.01 level (2-tailed) as shown in the Table 37c. Within 9 – 13 days the *Hylarana nicobariensis* tadpole intestine

(from stage 40 – stage 46) shortens in length by about 83%. At stage 46, the primary epithelium (PE) degenerates and the secondary epithelium (SE) are formed. These intestinal folds (IF) appear as several circular folds that run longitudinally and are straight along the gut axis, lined by mucus membrane (Fig. 4.112c&d).

***Kaloula pulchra*:** The length of tadpole intestine was increased from stage 25 to stage 40, there is significant positive correlation between the tadpole length and the gut length at the 0.01 level (2-tailed), where $p<0.01$ (Table 38b). Histological study revealed that the primary epithelia are surrounded by submucosa which is further enclosed by a thin serosa (Fig. 4.113a&b). The intestine attained its maximum length (62.75 mm) at stage 40 where the tadpole reached a maximum total length, 29.92 mm (Table 38a). From stage 40 to stage 46, Table 38c showed that the lengths of total body and intestines greatly reduced, showing positive correlation ($p=0.017$) at the 0.01 level (2-tailed). Within 9 – 12 days the *Kaloula pulchra* tadpole intestine (from stage 40 – stage 46) reduced its length by about 80%. At stage 46, the primary epithelium (PE) degenerates and the secondary epithelium (SE) are formed. Intestinal folds (IF) appear as several circular folds lined by mucus membrane (Fig. 4.113c&d).

***Microhyla berdmorei*:** The present study revealed that, in *Microhyla berdmorei* the length of tadpole intestine is increased from stage 25 to stage 40 (Table 39a). There is significant positive correlation between the tadpole length and the gut length, where $p<0.01$ at the 0.01 level (2-tailed) as shown in the Table 39b. Histological study revealed that the intestine at these stages is a long simple tube with a single layer of cuboidal epithelial cells and the primary epithelia are surrounded by

submucosa which is further enclosed by a thin serosa (Fig. 4.114a&b). The intestine attained its maximum length (48.53 mm) at stage 40 where the tadpole reached a maximum total length, 25.24 mm. From stage 40 to stage 46, the lengths of total body and intestines greatly reduced, showing positive correlation at the 0.05 level, where $p=0.022$ (Table 39c). Within 10 – 17 days the *Microhyla berdmorei* tadpole intestine (from stage 40 – stage 46) reduced its length by about 82%. At stage 46, the primary epithelium (PE) degenerates and the secondary epithelium (SE) are formed. Intestinal folds (IF) appear as several circular folds lined by mucus membrane (Fig. 4.114c&d).

Table 1: Surveyed areas and sites of collections of selected species.

Sl. No.	Districts	Areas	Latitudes	Longitudes	Elevation (m asl)	1	2	3	4
1.	Aizawl	Aizawl	23°43'-44'N	92° 40'-44'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 (lake)	23°44'N	92°57'E	745	+	+	-	-
		Durlui stream	23°53'N	92°39'E	103	+	+	-	+
		Tuivawl river	23°36'N	93°01'E	488	+	+	-	+
		Tuirial river	23°43'N	92°47'E	179	+	+	-	+
		Tuirini river	23°41'N	92°53'E	298	+	+	-	+
2.	Champhai	Champhai	23°31'-32'N	93°15 - 16'E	951-1460	+	+	+	+
		Tuichang river	23°32'N	93°06'E	603	+	+	-	+
		Tuipui river	23°27'N	93°10'E	708	+	+	-	-
		Khawzawl	23°32'N	93°10'E	1158-1257	+	-	+	-
		Kawlkulh	23°43'N	93°05'E	965	+	-	-	-
		Khawhai	23°22'N	93°07'E	1458	+	-	-	-
3.	Kolasib	Bilkhawthlir	24°20'N	92°41'E	65	+	+	+	+
		Tuitun	23°58'N	92°41'E	308-326	+	+	+	+
		Kawnpui	23°56'N	92°41'E	910	+	+	+	-
		Buhchang	24°20'N	92°39'E	46	+	+	+	+
4.	Lawngtlai	Lawngtlai	22°31'N	92°53'E	847	+	+	+	+
5.	Lunglei	Theiriat	22°55'-56'N	92°45'E	1048-1060	+	+	-	+
		Ramlaitui	23°20'N	92°46'E	750	+	-	+	+
6.	Mamit	Tut river	23°46'N	92°31'E	74	+	+	-	+
		Lengpui	23°49'-50'N	92°37'E	390-400	+	+	+	+
		Zawlnuam	24°15'N	92°30'E	47	+	+	+	+
7.	Saiha	Khankawn	22°22'N	92°57'E	193	+	+	-	+
		New Latawh	22°22'N	92°55'E	458	+	+	-	-
8.	Serchhip	Chhingchhip	23°27'N	92°51'E	1113	+	+	-	-
		Mat river	23°27'N	92°50'E	651	+	+	+	+
		Thenzawl	23°17'N	92°46'E	741-810	+	+	+	+

1 = *Euphlyctis cyanophlyctis*; 2 = *Hylarana nicobarensis*; 3 = *Kaloula pulchra* ;
 4 = *Microhyla berdmorei* ; (-) = Absent; (+) = Present

Table 2: Morphometric measurements of male and female *Euphlyctis cyanophlyctis* (N= Total number of frogs examined).

Sl.No.	Characters	Males N=30		Females N=30	
		Range (mm)	Mean±SE	Range (mm)	Mean±SE
1	SVL	32.20 - 46.80	38.81 ±0.62	31.23 - 61.15	47.25 ±1.13
2	SL	5.0 - 8.60	6.07 ±0.12	5.01 - 10.24	6.99 ±0.19
3	EN	2.14 - 3.92	3.25 ±0.06	2.92 - 4.32	3.54 ±0.07
4	SN	2.18 - 3.74	2.77 ±0.08	2.13 - 4.25	2.95 ±0.10
5	T YE	0.75 - 1.36	1.08 ±0.03	1.0 - 2.06	1.41 ±0.06
6	INS	1.50 - 3.35	2.10 ±0.08	1.38 - 3.08	2.23 ±0.08
7	IOD	1.20 - 3.18	1.93 ±0.08	1.20 - 3.0	1.89 ±0.07
8	UE	2.06 - 4.70	3.35 ±0.10	2.88 - 4.86	3.52 ±0.07
9	ED	4.0 - 6.50	5.24 ±0.12	4.38 - 6.93	5.77 ±0.10
10	HTYD	3.20 - 5.86	4.41 ±0.12	3.24 - 6.38	4.71 ±0.14
11	VTYD	3.01 - 5.11	4.15 ±0.10	3.15 - 5.24	4.23 ±0.11
12	HL	10.60 - 14.70	12.67 ±0.23	12.0 - 19.20	15.49 ±0.34
13	HWN	5.04 - 7.70	5.95 ±0.13	5.12 - 8.04	7.13 ±0.14
14	HWAE	7.90 - 12.28	9.48 ±0.18	8.20 - 12.71	10.88 ±0.22
15	HWPE	12.25 - 17.42	14.01 ±0.19	14.05 - 18.21	15.62 ±0.22
16	HWAJ	12.0 - 16.30	14.28 ±0.25	13.0 - 22.0	16.97 ±0.38
17	HDN	3.87 - 6.20	5.11 ±0.11	4.82 - 6.92	6.04 ±0.10
18	HDE	6.12 - 8.81	7.97 ±0.11	7.68 - 10.91	9.27 ±0.19
19	HDAJ	7.05 - 11.68	10.13 ±0.20	9.07 - 14.45	11.25 ±0.26
20	MN	8.34 - 12.12	10.40 ±0.16	10.21 - 14.8	12.33 ±0.22
21	MAE	6.54 - 9.86	8.28 ±0.15	7.90 - 11.0	9.64 ±0.13
22	MPE	3.17 - 5.82	5.01 ±0.12	5.05 - 7.92	6.21 ±0.12
23	IAE	5.0 - 7.14	5.73 ±0.11	4.48 - 7.57	6.54 ±0.13
24	IPE	7.14 - 9.78	8.29 ±0.12	6.68 - 11.22	9.41 ±0.19
25	AG	12.14 - 19.20	15.24 ±0.35	14.20 - 25.36	19.30 ±0.52
26	FLL	15.07 - 25.50	20.90 ±0.43	21.03 - 31.34	24.93 ±0.55
27	HAL	7.0 - 12.0	10.02 ±0.19	7.80 - 19.41	11.23 ±0.49
28	F ₁	5.60 - 10.0	8.09 ±0.20	9.0 - 14.49	11.43 ±0.22
29	F ₂	5.0 - 9.93	7.63 ±0.23	8.70 - 13.0	10.40 ±0.20
30	F ₃	7.0 - 12.0	10.05 ±0.20	10.0 - 19.41	12.63 ±0.42
31	F ₄	5.40 - 10.50	8.23 ±0.23	7.50 - 14.49	11.16 ±0.26
32	F ₁ D	----	----	----	----
33	F ₃ D	----	----	----	----
34	HLL	51.0 - 72.60	59.93 ±1.01	56.0 - 90.0	71.23 ±1.56
35	TBL	14.03 - 21.50	18.49 ±0.33	18.0 - 29.85	22.96 ±0.47
36	TBW	4.71 - 6.75	5.94 ±0.07	5.11 - 13.50	6.87 ±0.28
37	TSL	8.0 - 11.24	9.53 ±0.17	8.60 - 16.21	12.33 ±0.37
38	FTL	16.50 - 23.0	20.48 ±0.29	19.0 - 28.13	24.33 ±0.44
39	T ₁	6.0 - 11.20	8.68 ±0.24	8.08 - 13.48	10.38 ±0.25
40	T ₂	10.0 - 15.0	12.09 ±0.26	11.42 - 17.95	14.72 ±0.30
41	T ₃	13.0 - 19.50	16.35 ±0.30	12.52 - 24.46	19.92 ±0.47
42	T ₄	16.50 - 23.0	20.48 ±0.29	19.0 - 28.13	24.33 ±0.44
43	T ₅	14.0 - 19.80	16.71 ±0.32	14.0 - 24.9	20.28 ±0.45
44	T ₁ D	----	--	-- --	----
45	T ₄ D	----	--	----	----

Table 3: Morphometric measurements of male and female *Hylarana nicobariensis*
(N= Total number of frogs examined).

Sl.No.	Characters	Males N=30		Females N=30	
		Range (mm)	Mean±SE	Range (mm)	Mean±SE
1	SVL	32.73 - 48.48	38.04 ±0.70	43.36 - 58.6	49.72 ±0.73
2	SL	5.15 - 8.18	6.47 ±0.14	6.69 - 9.45	7.87 ±0.13
3	EN	2.97 - 4.52	3.70 ±0.07	3.53 - 5.5	4.45 ±0.09
4	SN	1.92 - 3.64	2.73 ±0.08	2.39 - 4.12	3.27 ±0.07
5	T YE	0.54 - 2.74	1.28 ±0.09	0.83 - 2.74	1.79 ±0.08
6	INS	2.87 - 5.04	3.92 ±0.10	3.84 - 5.80	4.74 ±0.09
7	IOD	2.50 - 4.11	3.15 ±0.08	2.76 - 5.08	3.86 ±0.09
8	UE	5.21 - 7.20	6.39 ±0.08	6.30 - 8.30	7.03 ±0.08
9	ED	3.53 - 5.80	4.94 ±0.09	4.38 - 6.10	5.33 ±0.08
10	HTYD	2.91 - 4.50	3.68 ±0.07	3.21 - 4.78	3.86 ±0.08
11	VTYD	2.08 - 3.82	3.22 ±0.07	3.15 - 4.46	3.76 ±0.07
12	HL	11.12 - 17.40	12.92 ±0.25	12.46 - 18.44	15.54 ±0.23
13	HWN	4.18 - 6.84	5.38 ±0.11	5.24 - 8.12	6.41 ±0.13
14	HWAE	6.12 - 10.5	8.22 ±0.18	8.47 - 13.26	10.40 ±0.19
15	HWPE	9.70 - 14.40	11.65 ±0.22	10.8 - 17.46	14.37 ±0.25
16	HWAJ	10.36 - 15.60	12.37 ±0.25	13.1 - 19.22	15.41 ±0.26
17	HDN	2.44 - 6.60	3.92 ±0.16	3.64 - 6.6	5.02 ±0.12
18	HDE	4.42 - 8.62	6.65 ±0.17	4.42 - 9.72	8.11 ±0.18
19	HDAJ	5.70 - 10.62	7.62 ±0.21	7.37 - 12.66	9.09 ±0.24
20	MN	7.28 - 13.5	10.11 ±0.30	10.18 - 16.18	12.71 ±0.27
21	MAE	5.12 - 11.46	7.40 ±0.28	7.50 - 11.50	9.43 ±0.19
22	MPE	2.89 - 4.85	4.04 ±0.10	2.16 - 6.60	5.01 ±0.18
23	IAE	5.57 - 8.44	6.88 ±0.13	7.06 - 10.04	8.51 ±0.14
24	IPE	7.88 - 13	9.99 ±0.22	10.08 - 13.85	11.98 ±0.20
25	AG	11.16 - 23.8	16.69 ±0.54	14.12 - 30.86	21.74 ±0.65
26	FLL	17.0 - 26.79	22.71 ±0.47	22.6 - 36.02	27.99 ±0.60
27	HAL	7.30 - 12.67	10.02 ±0.21	10.29 - 14.28	12.40 ±0.20
28	F ₁	5.81 - 9.53	7.35 ±0.16	8.12 - 11.66	9.55 ±0.20
29	F ₂	4.50 - 9.06	6.84 ±0.18	7.08 - 10.77	8.82 ±0.18
30	F ₃	4.14 - 12.67	9.86 ±0.29	10.29 - 14.28	12.40 ±0.20
31	F ₄	6.44 - 10.30	8.36 ±0.16	8.68 - 12.50	10.40 ±0.21
32	F ₁ D	0.58 - 1.16	0.90 ±0.02	0.76 - 1.60	1.15 ±0.04
33	F ₃ D	0.72 - 1.40	1.03 ±0.03	0.84 - 1.68	1.28 ±0.04
34	HLL	57.02 - 82.94	69.09 ±1.34	73.55 - 107.3	86.94 ±1.60
35	TBL	19.33 - 33.24	24.53 ±0.58	25.8 - 37.04	29.77 ±0.57
36	TBW	2.43 - 8.81	4.14 ±0.25	3.77 - 7.52	5.42 ±0.16
37	TSL	7.41 - 17.52	12.21 ±0.38	12.12 - 18.86	15.03 ±0.31
38	FTL	16.28 - 28.51	20.97 ±0.53	21.12 - 30.52	25.71 ±0.51
39	T ₁	5.0 - 10.20	6.95 ±0.19	6.97 - 10.43	8.50 ±0.20
40	T ₂	7.80 - 14.04	10.33 ±0.26	10.06 - 15.6	12.84 ±0.28
41	T ₃	11.83 - 21.43	14.98 ±0.38	12.24 - 22.74	18.23 ±0.43
42	T ₄	16.28 - 28.5	20.97 ±0.52	21.12 - 30.52	25.57 ±0.49
43	T ₅	11.98 - 22.27	15.65 ±0.43	12.08 - 23.21	19.12 ±0.47
44	T ₁ D	0.63 - 1.56	1.00 ±0.04	1.02 - 1.56	1.29 ±0.03
45	T ₄ D	0.70 - 2.46	1.19 ±0.06	0.86 - 2.0	1.41 ±0.04

Table 4: Morphometric measurements of male and female *Kaloula pulchra*
(N= Total number of frogs examined).

Sl.No.	Characters	Males N=30		Females N=30	
		Range (mm)	Mean±SE	Range (mm)	Mean±SE
1	SVL	57.74 - 69.76	61.92 ± 0.49	59.90 - 69.94	64.60 ± 0.51
2	SL	6.79 - 8.80	7.36 ± 0.10	7.10 - 8.64	7.73 ± 0.09
3	EN	3.63 - 4.34	4.04 ± 0.05	3.57 - 4.57	4.12 ± 0.07
4	SN	2.06 - 3.12	2.73 ± 0.05	2.70 - 3.80	3.06 ± 0.06
5	T YE	1.07 - 2.98	1.81 ± 0.09	1.58 - 2.10	1.80 ± 0.04
6	INS	3.59 - 5.14	4.48 ± 0.09	4.16 - 4.93	4.63 ± 0.05
7	IOD	6.65 - 8.06	7.38 ± 0.09	7.10 - 8.28	7.60 ± 0.06
8	UE	3.42 - 4.90	3.89 ± 0.09	3.84 - 5.17	4.46 ± 0.09
9	ED	4.90 - 6.63	5.83 ± 0.10	5.65 - 7.75	6.52 ± 0.16
10	HTYD	3.75 - 4.56	4.13 ± 0.04	3.10 - 4.50	3.89 ± 0.09
11	VTYD	2.58 - 3.92	3.27 ± 0.10	3.08 - 3.63	3.41 ± 0.04
12	HL	12.46 - 16.47	14.19 ± 0.18	12.26 - 16.36	14.67 ± 0.27
13	HWN	5.03 - 7.60	6.37 ± 0.18	7.13 - 8.45	7.70 ± 0.09
14	HWAE	9.20 - 13.57	11.22 ± 0.28	9.77 - 13.66	12.66 ± 0.24
15	HWPE	16.76 - 21.37	18.67 ± 0.32	18.46 - 23.16	20.85 ± 0.26
16	HWAJ	20.22 - 23.78	21.37 ± 0.19	21.60 - 26.06	23.43 ± 0.29
17	HDN	4.44 - 6.78	6.14 ± 0.09	5.06 - 6.96	5.93 ± 0.11
18	HDE	8.22 - 10.34	9.21 ± 0.11	8.73 - 10.40	9.44 ± 0.09
19	HDAJ	10.17 - 15.55	12.41 ± 0.27	12.10 - 15.24	12.89 ± 0.16
20	MN	8.96 - 11.57	10.61 ± 0.15	10.14 - 11.54	10.99 ± 0.10
21	MAE	7.0 - 8.08	7.33 ± 0.05	6.97 - 8.55	7.67 ± 0.10
22	MPE	4.80 - 5.97	5.43 ± 0.07	4.56 - 6.64	5.56 ± 0.14
23	IAE	9.72 - 11.62	10.34 ± 0.13	10.0 - 12.25	10.95 ± 0.12
24	IPE	15.45 - 20.42	16.74 ± 0.22	16.62 - 23.75	18.70 ± 0.38
25	AG	16.26 - 27.42	21.82 ± 0.53	19.80 - 31.36	24.17 ± 0.69
26	FLL	32.07 - 42.58	38.58 ± 0.41	34.40 - 44.32	41.98 ± 0.60
27	HAL	9.86 - 20.87	18.79 ± 0.48	19.14 - 22.55	21.23 ± 0.19
28	F ₁	6.94 - 12.44	11.36 ± 0.19	10.84 - 13.90	12.17 ± 0.20
29	F ₂	6.63 - 16.15	14.84 ± 0.30	14.0 - 17.16	15.90 ± 0.22
30	F ₃	9.86 - 20.87	18.11 ± 0.54	19.14 - 22.55	21.23 ± 0.19
31	F ₄	8.07 - 17.50	16.44 ± 0.30	16.42 - 19.12	17.99 ± 0.14
32	F ₁ D	0.83 - 2.16	1.91 ± 0.04	1.98 - 2.50	2.23 ± 0.03
33	F ₃ D	0.88 - 3.08	2.67 ± 0.08	2.64 - 3.61	3.19 ± 0.05
34	HLL	67.34 - 83.62	73.52 ± 0.68	72.25 - 80.70	76.77 ± 0.53
35	TBL	22.36 - 25.40	23.48 ± 0.18	22.0 - 26.0	24.25 ± 0.25
36	TBW	3.17 - 9.32	7.69 ± 0.26	7.44 - 9.34	8.55 ± 0.11
37	TSL	11.62 - 14.02	12.83 ± 0.17	12.63 - 15.86	14.53 ± 0.20
38	FTL	19.90 - 69.76	25.53 ± 0.26	25.53 - 28.87	27.42 ± 0.20
39	T ₁	6.30 - 8.80	10.11 ± 0.21	10.0 - 10.90	10.50 ± 0.06
40	T ₂	9.55 - 4.34	14.09 ± 0.24	13.60 - 15.0	14.57 ± 0.07
41	T ₃	13.18 - 3.12	19.87 ± 0.29	18.88 - 21.86	20.67 ± 0.16
42	T ₄	19.9 - 2.98	25.53 ± 0.26	25.53 - 28.87	27.42 ± 0.20
43	T ₅	15.53 - 5.14	16.74 ± 0.14	16.10 - 19.24	17.65 ± 0.20
44	T ₁ D	0.85 - 8.06	1.54 ± 0.03	1.47 - 1.90	1.67 ± 0.03
45	T ₄ D	0.90 - 4.90	1.71 ± 0.05	1.98 - 2.33	2.05 ± 0.02

Table 5: Morphometric measurements of male and female *Microhyla berdmorei*
(N= Total number of frogs examined).

Sl.No.	Characters	Males N=30		Females N=30	
		Range (mm)	Mean±SE	Range (mm)	Mean±SE
1	SVL	28.55 - 33.88	31.07 ± 0.23	30.68 - 35.7	33.91 ± 0.28
2	SL	4.06 - 5.16	4.64 ± 0.06	4.22 - 5.25	4.65 ± 0.07
3	EN	2.0 - 3.81	2.69 ± 0.10	2.38 - 3.16	2.72 ± 0.06
4	SN	1.48 - 2.88	1.92 ± 0.08	1.4 - 2.85	1.97 ± 0.09
5	T YE	0.68 - 1.30	0.96 ± 0.03	0.75 - 1.33	1.08 ± 0.04
6	INS	2.18 - 3.41	2.61 ± 0.07	2.3 - 2.96	2.72 ± 0.03
7	IOD	2.04 - 3.38	2.80 ± 0.07	2.8 - 3.88	3.21 ± 0.06
8	UE	1.61 - 2.40	2.06 ± 0.05	1.88 - 2.83	2.36 ± 0.055
9	ED	2.07 - 4.17	3.35 ± 0.09	3.35 - 4.96	3.94 ± 0.08
10	HTYD	2.11 - 3.08	2.48 ± 0.04	2.23 - 3.27	2.60 ± 0.06
11	VTYD	2.0 - 2.81	2.43 ± 0.04	2.12 - 3.74	2.55 ± 0.09
12	HL	7.0 - 10.34	8.95 ± 0.20	7.56 - 10.81	8.63 ± 0.19
13	HWN	2.40 - 4.32	3.57 ± 0.10	3.7 - 4.66	4.12 ± 0.05
14	HWAE	5.47 - 6.78	6.10 ± 0.07	6.13 - 7.42	6.54 ± 0.06
15	HWPE	8.58 - 10.94	9.46 ± 0.11	8.6 - 10.51	9.49 ± 0.10
16	HWAJ	10.03 - 12.34	11.04 ± 0.11	9.85 - 13.07	11.09 ± 0.18
17	HDN	2.22 - 4.49	3.40 ± 0.09	3.08 - 4.3	3.44 ± 0.07
18	HDE	5.12 - 6.56	5.68 ± 0.06	4.82 - 6.5	5.59 ± 0.10
19	HDAJ	5.86 - 8.25	6.96 ± 0.12	6.17 - 7.6	6.73 ± 0.08
20	MN	5.48 - 8.0	6.48 ± 0.14	5.03 - 7.3	6.21 ± 0.12
21	MAE	3.97 - 6.64	4.72 ± 0.11	3.36 - 5.16	4.20 ± 0.12
22	MPE	1.47 - 4.46	2.94 ± 0.13	2.27 - 3.62	2.86 ± 0.07
23	IAE	4.05 - 5.61	5.08 ± 0.08	5 - 6.2	5.55 ± 0.08
24	IPE	6.90 - 8.26	7.51 ± 0.09	7.52 - 8.2	7.79 ± 0.04
25	AG	10.48 - 13.42	11.89 ± 0.13	10.5 - 15.29	12.69 ± 0.24
26	FLL	16.62 - 19.26	17.97 ± 0.17	14.6 - 21.14	19.10 ± 0.33
27	HAL	7.87 - 10.39	8.39 ± 0.11	7.48 - 9.88	8.78 ± 0.12
28	F ₁	3.30 - 4.95	3.95 ± 0.08	2.9 - 4.8	3.58 ± 0.10
29	F ₂	5.0 - 6.26	5.69 ± 0.07	5.31 - 6.2	5.77 ± 0.06
30	F ₃	7.87 - 10.39	8.39 ± 0.11	7.48 - 9.88	8.78 ± 0.12
31	F ₄	2.26 - 7.28	6.11 ± 0.21	6.24 - 6.94	6.48 ± 0.05
32	F ₁ D	0.31 - 0.93	0.58 ± 0.02	0.54 - 1.07	0.74 ± 0.02
33	F ₃ D	0.44 - 0.94	0.66 ± 0.03	0.6 - 1.7	0.88 ± 0.05
34	HLL	54.43 - 67.33	60.64 ± 0.71	56.1 - 69.78	64.06 ± 0.69
35	TBL	19.49 - 23.54	21.39 ± 0.23	20.7 - 25.34	23.42 ± 0.31
36	TBW	4.18 - 5.25	4.45 ± 0.05	4.12 - 6.26	5.19 ± 0.15
37	TSL	7.30 - 11.30	9.51 ± 0.23	9.14 - 11.21	9.94 ± 0.14
38	FTL	16.33 - 20.38	18.63 ± 0.22	18.25 - 22.92	19.74 ± 0.20
39	T ₁	5.56 - 7.92	6.76 ± 0.12	5.66 - 7.18	6.39 ± 0.09
40	T ₂	8.87 - 11.84	10.06 ± 0.16	9.3 - 11.77	10.30 ± 0.14
41	T ₃	13.12 - 17.14	14.85 ± 0.21	13.6 - 17.65	15.23 ± 0.22
42	T ₄	16.33 - 20.38	18.63 ± 0.22	18.25 - 22.92	19.71 ± 0.20
43	T ₅	14.10 - 16.55	15.37 ± 0.15	14.4 - 18.38	15.86 ± 0.22
44	T ₁ D	0.26 - 1.05	0.69 ± 0.04	0.53 - 1.34	1.03 ± 0.04
45	T ₄ D	0.70 - 1.52	1.03 ± 0.04	0.73 - 2.03	1.41 ± 0.06

Table 6: Atmospheric temperature, relative humidity, and rain fall at study site-I, Kawnpui, Kolasib District during 2005-2007.

Months	Temperature(°C)						Relative humidity (%)						Rainfall (mm)		
	2005		2006		2007		2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max			
Jan	11	25.5	13	24.5	10.5	18	47	87	63	88	56	84	0	0	0
Feb	19	29	17	28	11	19	47	77	52	86	45	81	14	0	57.1
Mar	19	33	18	32	15	24.5	48	85	47	85	51	85	228.42	1.2	30.4
Apr	21	36	21	31	15	22.5	44	91	46	88	54	85	129.3	264.7	360.8
May	21	31	20	32	23	28.6	45	85	47	91	56	88	435.2	462.1	342.2
Jun	25	31.5	22	32	20	32.5	53	91	62	89	62	90	121	673.1	420.7
Jul	24	30	23	33	21.5	31	51	95	65	92	64	92	704	457.1	376
Aug	25	34.5	24	31	22	30.5	44.5	87	67	91	58	88	332.7	415.4	600.1
Sep	24	29	24	30	24	32	42	86	63	92	53	85	383.8	214.4	611.4
Oct	21.5	24	17	26	18	30.5	48	85	57	89	60	89	189.4	224.8	269.3
Nov	14	25	14	22	15	28	56	87	58	94	62	87	0	8.3	11
Dec	13.5	23	13	18.5	13	21.5	61	91	61	89	68	88	0	0	0

Table 7: Ecological parameters (water temperature and pH) at study site I, Kawnpui (Kolasib District), during 2005 - 2007

Months	Water temperature (°C)						pH		
	2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max			
Jan	15	18	15.5	19	11	12.5	7.1±0.66	7.0±0.53	7.1±0.36
Feb	21	25	18	23	11	13	7.2±0.45	7.1±0.46	7.2±0.24
Mar	22	28	22	27	18	22.5	6.8±0.66	7.2±0.53	7.0±0.36
Apr	26	30	25	30	23.5	26	6.5±0.45	7.2±0.46	6.6±0.24
May	25	31	25.5	30	24	31.5	7.0±0.28	6.9±0.13	6.5±0.22
Jun	26	32	27	33	26	31	6.9±0.39	6.7±0.25	6.7±0.12
Jul	27.5	29.5	25	33	24	29.5	6.8±0.56	6.4±0.22	6.6±0.13
Aug	25	29	23	29	24	29	6.7±0.28	6.5±0.70	6.6±0.19
Sep	23	26	22	27	23	25	6.5±0.12	6.6±0.63	6.6±0.56
Oct	18	23	16	23	23	28	6.5±0.19	6.8±0.12	6.5±0.45
Nov	15	19.5	13.5	18.5	23	25.5	6.7±0.66	7.0±0.53	6.4±0.36
Dec	14	18	14	17	15	17	7.0±0.45	7.1±0.46	6.7±0.24

Table 8: Atmospheric temperature, relative humidity, and rain fall at study site- II, Kawnpui,
Kolasib District during 2005-2007.

Months	Temperature(°C)						Relative humidity (%)						Rainfall (mm)		
	2005		2006		2007		2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max			
Jan	10	24.5	12	24	10.5	17	51	88	47	87	48	86	0	0	0
Feb	17	27	16	26	11	18	53	87	56	84	48	87	14	0	57.1
Mar	18	32	17.5	31	14	23	55	87	57	87	56	84	228.42	1.2	30.4
Apr	21	34	20.5	33	17	28.5	54	90	58	92	58	87	129.3	264.7	360.8
May	21.5	30	22	30	22	29.5	56	88	62	91	57	92	435.2	462.1	342.2
Jun	24	32	23	31	20	32	61	92	65	93	61	91	121	673.1	420.7
Jul	23.5	31	23	32	22.5	31.5	62	96	64	92	62	92	704	457.1	376
Aug	25.5	32	24.5	31	23	32.5	68.5	87	68	92	59	93	332.7	415.4	600.1
Sep	24	29.5	23	30	23.5	33	56	86	61	91	58	87	383.8	214.4	611.4
Oct	20.5	26	19	28	18	29.5	55	85	58	89	60	91	189.4	224.8	269.3
Nov	13.5	25.5	14	27	15	28.5	53	89	55	91	62	88	0	8.3	11
Dec	12.5	23	13	18.5	13	21.5	52	91	52	89	50	87	0	0	0

Table 9: Ecological parameters (water temperature and pH) at study site II, Kawnpui (Kolasib District), during 2005 - 2007

Months	Water temperature (°C)						pH		
	2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max			
Jan	13	16	13.5	15	12	15	6.4±0.14	6.4±0.22	6.3±0.12
Feb	17	20	13.5	18	12.5	19	6.7±0.15	6.5±0.36	6.3±0.44
Mar	20	26	20.5	27	18.5	21.5	6.8±0.61	7.1±0.24	6.6±0.12
Apr	21.5	27	22	27.5	20.5	25.5	6.5±0.25	7.1±0.13	6.7±0.23
May	22.5	27.5	21.5	28	23	28.5	6.8±0.18	6.8±0.34	6.5±0.56
Jun	25	28	23	28	23	27.5	7.1±0.32	6.5±0.24	6.8±0.42
Jul	26.5	29	25	28.5	24.5	28.5	6.7±0.56	6.4±0.43	6.6±0.45
Aug	23.5	27	22	28	22	27.5	6.4±0.28	6.6±0.56	6.5±0.23
Sep	22	26.5	22.5	26	22.5	25.5	6.6±0.14	6.7±0.34	6.6±0.33
Oct	17	21.5	16.5	23.5	17.5	22	6.5±0.23	6.8±0.18	6.5±0.24
Nov	16.5	19.5	14.5	20.5	16	21.5	6.8±0.15	6.6±0.32	6.5±0.45
Dec	14.5	18.5	15	18	14	17	6.8±0.32	6.4±0.54	6.7±0.58

Table 10: Atmospheric temperature, relative humidity and rain fall at study site-III, Sihhmui, Aizawl district during 2005-2007.

Months	Temperature(°C)						Relative humidity (%)						Rainfall (mm)		
	2005		2006		2007		2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max			
Jan	10	26	12	28	10	27	51	88	48	78	53	78	12	0	0
Feb	14	27	15	26	14	27.5	52	84	52	86	56	79	10	0	56
Mar	15	32	18.5	32	16	29.5	53	92	47	85	57	79	122	6	24
Apr	24	32.5	22	29	19.5	31.5	48	86	48	86	52	90	58	49	331
May	25.5	33	23.5	30	24	26.5	49	86	52	88	53	93	432	423	241
Jun	21.5	32	19.5	31	19	29.5	50	96	51	92	56	98	148	407	525
Jul	14.5	28	18	32	21.5	31	54	95	56	94	61	94	594	426	201
Aug	19	28.5	17	32	22.5	32.5	60	92	54	88	65	97	266	329	350
Sep	23.5	35.5	21	31	21	33	54	89	55	91	62	95	372	328	330
Oct	22	35	17.5	31	24.5	32	51	92	53	86	57	96	246	67	162
Nov	18	32	15	29	16.5	30	48	91	54	86	52	93	34	11	69
Dec	11	27.5	12	27	11	28	47	90	46	84	48	88	0	0	0

Table 11: Ecological parameters (water temperature and pH) at study site III, Sihhmui (Aizawl District), during 2005- 2007

Months	Water temperature (°C)						pH		
	2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max			
Jan	*	*	*	*	*	*	*	*	*
Feb	*	*	*	*	*	*	*	*	*
Mar	15.5	23	18.5	22	15	23.5	6.2±0.43	6.2±0.56	6.0±0.33
Apr	23	27.5	21	26	24	28	6.4±0.32	6.2±0.67	6.3±0.76
May	19.5	26	20.5	27	20	26.5	6.6±0.25	6.7±0.65	6.4±0.42
Jun	18.5	25.5	19.5	25	21	25.5	6.5±0.44	6.6±0.56	6.5±0.55
Jul	19.5	26	18	25	21.5	25	6.7±0.52	6.4±0.28	6.6±0.72
Aug	18	24.5	19.5	24.5	18.5	25.5	6.4±0.28	6.5±0.73	6.7±0.40
Sep	19.5	25.5	21	23	17	24	6.4±0.34	6.6±0.54	6.6±0.55
Oct	20	23.5	17.5	24.5	18.5	24	6.6±0.14	6.8±0.16	6.5±0.64
Nov	18.5	20.5	15	29	16.5	20	6.4±0.43	7.0±0.58	6.4±0.45
Dec	13	17.5	12.5	17	11.5	18	6.3±0.26	6.1±0.14	6.7±0.54

* = No water

Table 12: Atmospheric temperature, relative humidity and rainfall at study site-IV, Sairang during 2005- 2007.

Months	Temperature(°C)						Relative humidity (%)						Rainfall (mm)		
	2005		2006		2007		2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max			
Jan	10.5	28.5	12	27.5	10.5	26.5	45	86	42	75	43	75	12	0	0
Feb	14.5	29	15.5	28	14.5	29	41	84	43	85	43	77	10	0	56
Mar	15	33.5	19	32.5	14	30	43	85	46	87	47	78	122	6	24
Apr	14.5	32.5	18	30	18.5	32	46	87	48	85	52	89	58	49	331
May	16.5	33.5	21	30.5	20.5	29	47	88	52	87	51	92	432	423	241
Jun	15.5	33	20.5	32	19	30	48	98	55	91	57	97	148	407	525
Jul	19.5	28	18.5	31.5	22	32	51	93	54	94	63	93	594	426	201
Aug	21	29	18	33	21.5	33	61	92	56	96	68	97	266	329	350
Sep	24	35.5	21	31.5	21	34	56	82	54	92	63	94	372	328	330
Oct	22.5	35	17.5	31.5	21	32.3	48	92	48	85	58	96	246	67	162
Nov	19	32	15.5	29.5	17	30.5	49	92	44	86	54	92	34	11	69
Dec	12.5	29	12.5	28	11.5	28.0	47	91	42	84	43	83	0	0	0

Table 13: Ecological parameters (water temperature and pH) at study site IV, Sairang, Aizawl District, during 2005- 2007

Months	Water temperature (°C)						pH		
	2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max			
Jan	13.5	19	13	18	15.5	20	7.4±0.26	7.2±0.56	7.3±0.52
Feb	14.5	19.5	17	21.5	17.5	21	7.2±0.34	7.3±0.43	7.1±0.66
Mar	17	22	18.5	23.5	19	24	6.4±0.33	6.8±0.38	6.9±0.32
Apr	24	26	22.5	26	18.5	26.5	6.5±0.56	6.5±0.67	6.7±0.72
May	25.5	26	23.5	27	22	28.5	6.4±0.54	7.4±0.65	6.8±0.44
Jun	26	29	24.5	28	24	26.5	6.9±0.67	7.6±0.54	7.2±0.35
Jul	18	28	21	27.5	20.5	27	7.5±0.42	6.8±0.48	7.6±0.42
Aug	20	27	19.5	26.5	19.5	26.5	7.1±0.34	6.8±0.53	6.7±0.45
Sep	24	25	21.5	25	20	25	7.5±0.48	7.6±0.65	6.8±0.50
Oct	16	24	17	23.5	17.5	24.5	6.9±0.46	6.8±0.53	6.5±0.62
Nov	16	19.5	16.5	20.5	17	20.5	7.4±0.36	7.2±0.42	6.7±0.41
Dec	15.5	19	14.5	19.5	16.5	18	7.2±0.48	6.9±0.64	6.7±0.54

Table 14: Atmospheric temperature, relative humidity and rain fall at study site-V, Tanhril, Aizawl district during 2005-2007.

Months	Temperature(°C)						Relative humidity (%)						Rainfall (mm)		
	2005		2006		2007		2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max			
Jan	11.5	24.5	12.5	25.5	11.5	24	42	82	48	73	46	73	8	0	0
Feb	14	27.5	14.5	28.5	13.5	27	48	82	49	86	54	84	0	6	39.1
Mar	15	30.5	15.5	30.5	15.5	29.5	51	86	52	91	52	89	156.6	3	31
Apr	15.5	32	14.5	30.5	17.5	31	48	87	53	83	57	88	120.9	60.5	285.9
May	16	31	15.5	31.5	17	32.5	47	88	57	86	54	87	425.9	539.7	293.5
Jun	15	29.5	14.5	30.5	15.5	30	50	89	53	85	56	89	83.5	384	362.7
Jul	16.5	28	15.5	29	15	27.5	54	91	53	92	57	91	449.2	293.7	374
Aug	18	28.5	22.5	29	19.5	29	61	92	51	88	51	89	257.9	216.5	432.7
Sep	18.5	29	22	28	20.5	26	58	93	49	83	54	95	342.3	222.6	612.1
Oct	16	28	21	28.5	20.5	27.5	48	88	48	86	53	92	155.2	113.3	171.2
Nov	15	27	14.5	28.5	16.5	28	47	78	43	83	47	84	114	0	125
Dec	12.5	26	12	27.5	12.5	27	45	74	45	72	45	76	2	0	0

Table 15: Ecological parameters (water temperature and pH) at study site V, Tanhril (Aizawl District), during 2005- 2007

Months	Water temperature (°C)						pH		
	2005		2006		2007		2005	2006	2007
	Min	Max	Min	Max	Min	Max			
Jan	*	*	*	*	*	*	*	*	*
Feb	*	*	*	*	14.5	26	*	*	6.1±0.46
Mar	15.5	28.5	*	*	14.5	28.5	6.2±0.34	*	6.4±0.23
Apr	15	29	14.5	27.5	15.5	28	6.4±0.65	6.3±0.36	6.5±0.42
May	16.5	28	15.5	28.5	17	25.5	6.4±0.54	6.4±0.35	6.7±0.46
Jun	15.5	27.5	14.5	28	15.5	26	6.8±0.67	6.7±0.45	7.2±0.35
Jul	17.5	28	14.5	29	15	27.5	7.2±0.32	6.8±0.48	7.3±0.42
Aug	18.5	28.5	17.5	27	19.5	29	7.2±0.44	6.9±0.43	6.7±0.54
Sep	19.5	29	19	27.5	18.5	24.5	7.1±0.48	6.6±0.52	7.1±0.52
Oct	19	28	20	27.5	17.5	26.5	6.8±0.47	6.5±0.25	6.5±0.53
Nov	15	26.5	13.5	28	15.5	27	6.6±0.36	6.2±0.42	6.7±0.14
Dec	11.5	25	11.5	26.5	12	24	6.2±0.44	6.4±0.46	6.5±0.34

* = No water

Table 16(a): ANOVA table to test the significance of difference between air temperature at the study site-I during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	252.2803	1	252.2803	20.552	0.000
	Within Groups	417.3503	34	12.27501		
	Total	669.6306	35			

Table 16(b): ANOVA table to test the significance of difference between relative humidity at the study site-I during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	0.015625	1	0.015625	0.001	0.980
	Within Groups	820.8368	34	24.14226		
	Total	820.8524	35			

Table 16(c): ANOVA table to test the significance of difference between rainfall at the study site-I during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	530692.8	1	530692.8	15.138	0.000
	Within Groups	1191918	34	35056.4		
	Total	1722610	35			

Table 16(d): ANOVA table to test the significance of difference between water temperature at the study site-I during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	8.333	25	0.333	5	0.006
	Within Groups	0.667	10	0.067		
	Total	9	35			

Table 16(e): ANOVA table to test the significance of difference between pH of water at the study site-I during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	1.733	8	0.217	0.805	0.604
	Within Groups	7.267	27	0.269		
	Total	9	35			

Table 17(a): ANOVA table to test the significance of difference between air temperature at the study site-IV during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	24.75062	1	24.75062	3.676	0.064
	Within Groups	228.9018	34	6.732406		
	Total	253.6524	35			

Table 17(b): ANOVA table to test the significance of difference between relative humidity at the study site-IV during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	5.25	23	0.228261	0.730	0.751
	Within Groups	3.75	12	0.3125		
	Total	9	35			

Table 17(c): ANOVA table to test the significance of difference between rainfall at the study site-IV during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	290700.7	1	290700.7	11.794	0.002
	Within Groups	838021.6	34	24647.69		
	Total	1128722	35			

Table 17(d): ANOVA table to test the significance of difference between water temperature at the study site-IV during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	8	24	0.333333	3.667	0.014
	Within Groups	1	11	0.090909		
	Total	9	35			

Table 17(e): ANOVA table to test the significance of difference between pH of water at the study site-IV during breeding and non-breeding seasons of *Euphlyctis cyanophlyctis*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	1.866667	10	0.186667	0.654	0.754
	Within Groups	7.133333	25	0.285333		
	Total	9	35			

Table 18(a): ANOVA table to test the significance of difference between air temperature at the study site-II during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	8.5	28	0.303571	4.250	0.027
	Within Groups	0.5	7	0.071429		
	Total	9	35			

Table 18(b): ANOVA table to test the significance of difference between relative humidity at the study site-II during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	164.125	1	164.125	19.752	0.000
	Within Groups	282.519	34	8.309384		
	Total	446.6441	35			

Table 18(c): ANOVA table to test the significance of difference between rainfall at the study site-II during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	833286.9	1	833286.9	31.858	0.000
	Within Groups	889323.5	34	26156.57		
	Total	1722610	35			

Table 18(d): ANOVA table to test the significance of difference between water temperature at the study site-II during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	8.25	24	0.34375	7.563	0.001
	Within Groups	0.5	11	0.045455		
	Total	8.75	35			

Table 18(e): ANOVA table to test the significance of difference between pH of water at the study site-II during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	0.169175	1	0.169175	4.283	0.046
	Within Groups	1.343048	34	0.039501		
	Total	1.512222	35			

Table 19(a): ANOVA table to test the significance of difference between air temperature at the study site-IV during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	77.97691	1	77.97691	15.091	0.000
	Within Groups	175.6755	34	5.166927		
	Total	253.6524	35			

Table 19(b): ANOVA table to test the significance of difference between relative humidity at the study site-IV during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	317.0032	1	317.0032	11.587	0.002
	Within Groups	930.219	34	27.35938		
	Total	1247.222	35			

Table 19(c): ANOVA table to test the significance of difference between rainfall at the study site-IV during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	290700.7	1	290700.7	11.794	0.002
	Within Groups	838021.6	34	24647.69		
	Total	1128722	35			

Table 19(d): ANOVA table to test the significance of difference between water temperature at the study site-IV during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	8.25	24	0.34375	7.563	0.001
	Within Groups	0.5	11	0.045455		
	Total	8.75	35			

Table 19(e): ANOVA table to test the significance of difference between pH of water at the study site-IV during breeding and non-breeding seasons of *Hylarana nicobariensis*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	0.052071	1	0.052071	0.392	0.535
	Within Groups	4.515429	34	0.132807		
	Total	4.5675	35			

Table 20(a): ANOVA table to test the significance of difference between air temperature at the study site-III during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	2.920139	1	2.920139	0.274	0.604
	Within Groups	362.4323	34	10.65977		
	Total	365.3524	35			

Table 20(b): ANOVA table to test the significance of difference between relative humidity at the study site-III during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	5.166667	22	0.234848	1.078	0.458
	Within Groups	2.833333	13	0.217949		
	Total	8	35			

Table 20(c): ANOVA table to test the significance of difference between rainfall at the study site-III during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	26182.35	1	26182.35	0.807	0.375
	Within Groups	1102540	34	32427.65		
	Total	1128722	35			

Table 20(d): ANOVA table to test the significance of difference between water temperature at the study site-III during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	5.133333	19	0.270175	2.316	0.087
	Within Groups	1.166667	10	0.116667		
	Total	6.3	29			

Table 20(e): ANOVA table to test the significance of difference between pH of water at the study site-III during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	2.788095	9	0.309788	1.764	0.139
	Within Groups	3.511905	20	0.175595		
	Total	6.3	29			

Table 21(a): ANOVA table to test the significance of difference between air temperature at the study site-V during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	Df	Mean Square	F	p
Air Temperature	Between Groups	4.833333	22	0.219697	0.902	0.598
	Within Groups	3.166667	13	0.24359		
	Total	8	35			

Table 21(b): ANOVA table to test the significance of difference between relative humidity at the study site-V during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	6.2	22	0.281818	2.035	0.094
	Within Groups	1.8	13	0.138462		
	Total	8	35			

Table 21(c): ANOVA table to test the significance of difference between rainfall at the study site-V during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	8793.59	1	8793.59	0.275	0.603
	Within Groups	1087655	34	31989.85		
	Total	1096449	35			

Table 21(d): ANOVA table to test the significance of difference between water temperature at the study site-V during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	0.114683	1	0.114683	0.048	0.828
	Within Groups	67.08532	28	2.395904		
	Total	67.2	29			

Table 21(e): ANOVA table to test the significance of difference between pH of water at the study site-V during breeding and non-breeding seasons of *Kaloula pulchra*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	3.333333	11	0.30303	1.839	0.121
	Within Groups	2.966667	18	0.164815		
	Total	6.3	29			

Table 22(a): ANOVA table to test the significance of difference between air temperature at the study site-II during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	439.2517	1	439.2517	64.299	0.000
	Within Groups	232.2674	34	6.831393		
	Total	671.5191	35			

Table 22(b): ANOVA table to test the significance of difference between relative humidity at the study site-II during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	218.7934	1	218.7934	32.648	0.000
	Within Groups	227.8507	34	6.701491		
	Total	446.6441	35			

Table 22(c): ANOVA table to test the significance of difference between rainfall at the study site-II during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	1092052.867	1	1092053	58.884	0.000
	Within Groups	630557.4727	34	18545.81		
	Total	1722610.34	35			

Table 22(d): ANOVA table to test the significance of difference between water temperature at the study site-II during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	8.5	24	0.354167	7.792	0.001
	Within Groups	0.5	11	0.045455		
	Total	9	35			

Table 22(e): ANOVA table to test the significance of difference between pH of water at the study site-II during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	0.885714	6	0.147619	0.528	0.783
	Within Groups	8.114286	29	0.279803		
	Total	9	35			

Table 23(a): ANOVA table to test the significance of difference between air temperature at the study site-IV during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Air temperature	Between Groups	77.58674	1	77.58674	14.983	0.000
	Within Groups	176.0657	34	5.178403		
	Total	253.6524	35			

Table 23(b): ANOVA table to test the significance of difference between relative humidity at the study site-IV during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Relative humidity	Between Groups	506.25	1	506.25	23.230	0.000
	Within Groups	740.9722	34	21.7933		
	Total	1247.222	35			

Table 23(c): ANOVA table to test the significance of difference between rainfall at the study site-IV during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Rainfall	Between Groups	691946.7	1	691946.7	53.863	0.000
	Within Groups	436775.6	34	12846.34		
	Total	1128722	35			

Table 23(d): ANOVA table to test the significance of difference between water temperature at the study site-IV during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Water temperature	Between Groups	294.6944	1	294.6944	118.410	0.000
	Within Groups	84.61806	34	2.488766		
	Total	379.3125	35			

Table 23(e): ANOVA table to test the significance of difference between pH of water at the study site-IV during breeding and non-breeding seasons of *Microhyla berdmorei*

		Sum of Squares	df	Mean Square	F	p
Water pH	Between Groups	0.006944	1	0.006944	0.052	0.821
	Within Groups	4.560556	34	0.134134		
	Total	4.5675	35			

Table 24: Size of amplexing pairs and clutch size of *Euphlyctis cyanophlyctis* during the study period (2005 – 2007)

Sl No.	2005			2006			2007		
	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size
1	38.23	51.25	153	36.83	45.54	175	43.8	59.2	138
2	42.15	43.2	75	38.63	47.64	156	40	54.32	152
3	34.25	51.2	102	41	46.58	161	37.34	57.8	169
4	46.8	50.15	151	35.7	45.34	97	41.32	56	175
5	39.17	45.24	82	37.65	45.16	104	42.42	59.3	145
6	37.25	40.32	167	41.34	59.2	169	42.56	61.4	137
7	35.42	31.23	145	37.12	56	113	39.34	60.12	96
8	40.12	35.64	163	45.8	55.56	182	36.5	54.2	169
9	41.23	50.2	93	36	51.32	178	37.4	48	178
10	35.62	42.4	98	42.5	47.4	126	43	54.6	142
11	39.5	36.3	87	41.45	59.2	167	44	53.54	173
12	32.2	54.32	146	42.67	60.12	118	42.7	59.3	168
13	38.56	61.15	68	39.3	57	169	38.4	54.24	131
14	38.56	53.2	95	38	53.4	155	41.2	58.3	148
15	41.57	48.25	115	33.65	45.6	83	39.43	48	169
16	41.63	47.18	126	36.5	51.73	156	43.23	49.6	104
17	40	45.46	143	43	57.12	143	39.4	54.12	167
18	41.29	56.39	162	37	55.12	102	43	49.34	98
19	40	49.17	132	38.24	54.7	78	37.65	59.4	173
20	38.83	49.5	154	39.2	59	126	39.64	56.6	157

Table 25: Size of amplexing pairs and clutch size of *Hylarana nicobariensis* during the study period (2005 – 2007)

Sl No.	2005			2006			2007		
	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size
1	39.5	50.76	514	37.12	48.48	507	40.82	54.25	315
2	39.7	45.92	275	37.4	49.47	615	40.98	52.34	512
3	38.38	43.36	210	42.61	44.85	467	38.45	55.82	619
4	41.64	44.89	587	48.48	49.66	479	42.33	56.43	515
5	34.72	52.3	529	44.89	45.76	143	42.24	54.33	405
6	44.89	49.47	617	40.35	56.43	612	43.65	47.42	317
7	35.85	44.85	514	38.14	56.34	312	38.64	50.09	165
8	36.07	52.66	136	35.83	57.65	218	46.51	51.25	147
9	37.86	42.36	131	43.69	52.23	478	38.48	49.05	518
10	33.82	51.57	498	44.28	55.42	612	32.34	45.4	412
11	32.73	55.86	578	42.46	54.24	617	45.47	51.45	317
12	35.68	55.74	416	43.68	58.21	181	42.34	51.36	613
13	32.88	46.3	628	43.92	57.78	619	39.41	52.26	438
14	34	48.36	597	38	55.45	514	34.12	48.34	418
15	36.58	49.26	151	33.65	55.65	387	34.42	48.9	196
16	35.28	56.32	612	36.5	54.37	613	44.32	49.68	140
17	37.85	58.6	413	43	55.21	415	39.48	45.21	176
18	38.22	45.34	152	37	56.12	210	44.43	49.43	189
19	35.04	53.94	604	38.24	54.73	187	36.57	55.64	317
20	34.08	51.27	131	39.2	52.96	617	35.68	54.62	512

Table 26: Size of amplexing pairs and clutch size of *Kaloula pulchra* during the study period (2005 – 2007)

Sl No.	2005			2006			2007		
	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size
1	69.76	69.94	478	61.84	62.32	515	59.43	63.25	471
2	61.57	69.73	541	62.56	64.53	438	63.24	65.47	538
3	59.7	59.9	534	63.58	67.71	478	63.26	64.58	429
4	61.6	64.23	427	59.38	61.23	487	59.84	61.59	435
5	65.9	67.9	488	62.8	67.56	407	62.12	63.27	432
6	57.74	61.32	527	67.56	68.23	465	62.65	64.36	476
7	60.34	62.56	448	62.96	63.25	519	63.05	65.32	576
8	62.31	69.73	576	62.14	65.22	480	58.04	61.35	452
9	59.88	64.23	498	61.33	64.52	506	59.35	62.48	406
10	58.97	62.12	547	60.44	63.47	486	58.97	62.52	548
11	61.56	61.93	459	61.48	63.25	406	61.55	63.45	425
12	65.89	68.42	487	62.48	63.44	501	60.48	62.54	363
13	57.95	63.23	554	61.95	63.35	477	59.38	62.46	544
14	-	-	-	60.83	62.34	365	61.76	64.57	479
15	-	-	-	58.36	62.56	434	63.68	65.48	546
16	-	-	-	61.58	63.45	531	67.35	69.96	553
17	-	-	-	59.26	62.38	508	60.96	63.51	432
18	-	-	-	-	-	-	59.34	62.43	517
19	-	-	-	-	-	-	59.48	61.49	467
20	-	-	-	-	-	-	63.45	65.35	459

Table 27: Size of amplexing pairs and clutch size of *Microhyla berdmorei* during the study period (2005 – 2007)

Sl No.	2005			2006			2007		
	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size	SVL male (mm)	SVL female (mm)	Clutch size
1	32.05	35.45	360	31.34	35.34	435	29.35	32.54	384
2	30.7	34.9	443	29.62	33.85	356	32.43	35.42	435
3	32.8	32.68	314	30.56	34.36	365	32.7	34.57	294
4	30.1	31.02	270	31.32	32.88	417	28.81	31.56	345
5	31.5	33.97	422	30.82	35.34	308	31.2	32.58	435
6	30.16	35.7	224	28.45	31.92	406	29.62	31.6	373
7	31.3	34.83	345	29	32.56	311	30.9	32.62	299
8	31	34.6	263	32.45	35.53	382	30.45	33.52	432
9	29.32	32.08	394	31.36	34.51	417	30.7	34.82	317
10	30.56	35.64	348	30.42	34.74	286	28.98	32.53	249
11	30.91	34.29	258	31.41	32.58	416	31.32	34.33	417
12	33.88	34.23	248	32.42	34.64	411	30.42	32.53	316
13	32.95	33.45	367	31.29	33.57	279	30.82	32.56	441
14	28.55	34.45	395	30.8	34.56	356	31.56	34.56	218
15	31.16	35.34	311	33.56	34.58	437	33.64	35.45	416
16	33.37	34.42	265	31.86	34.51	315	32.3	34.92	309
17	29.54	34.37	243	29.24	32.58	403	30.92	33.52	423
18	31.46	35.27	266	32.57	35.52	402	29.3	32.45	368
19	31.31	34.43	335	30.8	33.54	387	29.34	31.53	427
20	32.23	34.16	415	32.19	34.56	286	30.9	33.54	415

Table 28: Age and size of developing *Euphlyctis cyanophlyctis* embryos reared at 21°C – 30°C. (N= Total number of samples examined).

Sl.No.	Stage	Age	Size in mm(N=10)
1.	Fertilization	0 hr	1.35 ±0.03
2.	Gray Crescent	0:25 hr	1.36 ±0.05
3.	2-cell	1:45 hr	1.36 ±0.06
4.	4-cell	2:20 hr	1.36 ±0.03
5.	8-cell	3:45 hr	1.36 ±0.02
6.	16-cell	5:15 hr	1.37 ±0.04
7.	32-cell	6:30 hr	1.36 ±0.05
8.	Mid Cleavage	8:30 hr	1.36 ±0.64
9.	Late Cleavage	10:15 hr	1.38 ±0.25
10.	Dorsal Lip	15:30 hr	1.38 ±0.01
11.	Yolk Plug	21:15 hr	1.39 ±0.02
12.	Late Gastrula	1 day 1:45 hr	1.45 ±0.06
13.	Neural Plate	1 day 6:30 hr	1.88 ±0.06
14.	Neural Fold	1 day 12:45 hr	1.96 ±0.25
15.	Rotation	1 day 15:30 hr	2.0 ±0.15
16.	Neural Tube	1 day 18:30 hr	2.14 ±0.09
17.	Tail Bud	1 day 23:45 hr	2.29 ±0.23
18.	Muscular Response	2 days 2 hr	3.50 ±0.62
19.	Heart Beat	2 days 6 hr	4.31 ±0.29
20.	Tail Elongation	3 days 3 hr	4.40 ±0.37
21.	Cornea Transparent	3 days 10 hr	5.43 ±0.19
22.	Tail Fin Circulation	3 day 21 hr	5.68 ±0.05
23.	Operculum present (Hatching stage)	4 days	5.99 ±0.47
24.	Left Gill	6 days	14.30 ±0.82
25.	Spiracles Forms	8 days	16.38 ±8.28
26.	$L < \frac{1}{2}D$	15 days	22.69 ±1.13
27.	$L \geq \frac{1}{2}D$	19 days	23.26 ±0.96
28.	$L \geq D$	22 days	25.0 ±1.13
29.	$L \geq 1\frac{1}{2}D$	25 days	29.0 ±3.98
30.	$L \geq 2D$	28 days	30.17 ±2.69
31.	Foot Paddle	30 days	31.07 ±1.41
32.	Indentation 4-5	32 days	31.75 ±3.18
33.	Indentation 3-4	34 days	36.35 ±0.21
34.	Indentation 2-3	36 days	37.60 ±0.99
35.	Indentation 1-2	37 days	40.27 ±1.42
36.	Toes 3-5 Separated	39 days	47.83 ±2.75
37.	All Toes Separated	41 days	47.40 ±0.14
38.	Metatarsal tubercles	43 days	58.25 ±4.59
39.	Sub-articular patches	45 days	57.90 ±4.46
40.	Foot Tubercles	47 days	64.60 ±6.64
41.	Fore Limbs Visible	51 days	64.50 ±4.95
42.	Fore Limbs emerge	59 days	60.0 ±2.83
43.	Tail Atrophies	61 days	53.95 ±0.07
44.	Tail Greatly Reduced	62 days	37.40 ±2.46
45.	Tail Stub	63 days	32.40 ±1.6
46.	Metamorphosis Complete	64 - 65 days	20.24 ±1.8

Table 29: Age and size of developing *Hylarana nicobarensis* embryos reared at 15°C – 25°C. (N= Total number of samples examined).

Sl.No.	Stage	Age	Size in mm (N=10)
1.	Fertilization	0 hr	1.59 ±0.01
2.	Gray Crescent	0:35 hr	1.75 ±0.02
3.	2-cell	1:40 hrs	1.64 ±0.01
4.	4-cell	2:45 hrs	1.75 ±0.02
5.	8-cell	4:25 hrs	1.76 ±0.03
6.	16-cell	6:25 hrs	1.78 ±0.01
7.	32-cell	9:20 hrs	1.78 ±0.08
8.	Mid Cleavage	13:45 hrs	1.87 ±0.01
9.	Late Cleavage	19:10 hrs	2.06 ±0.04
10.	Dorsal Lip	1 day	2.12 ±0.02
11.	Yolk Plug	1 day 6 hrs	2.0 ±0.06
12.	Late Gastrula	1 day 12 hrs	2.06 ±0.01
13.	Neural Plate	1 day 18 hrs	2.12 ±0.01
14.	Neural Fold	2 days	2.30 ±0.01
15.	Rotation	2 days 8 hrs	2.31 ±0.01
16.	Neural Tube	2 days 13 hrs	2.48 ±0.01
17.	Tail Bud	2 days 18 hrs	2.51 ±0.01
18.	Muscular Response	3 days 12 hrs	2.74 ±0.21
19.	Heart Beat	4 days 6 hrs	3.78 ±0.01
20.	Tail Elongation	5 days 4 hrs	5.76 ±0.11
21.	Cornea Transparent (Hatching stage)	6 days 8hrs	6.25 ±0.03
22.	Tail Fin Circulation	7 days	8.0 ±0.01
23.	Operculum present	10 days	8.23 ±0.12
24.	Left Gill	14 days	8.59 ±0.04
25.	Spiracles Forms	19 days	13.35 ±0.99
26.	$L < \frac{1}{2}D$	22 days	19.72 ±0.32
27.	$L \geq \frac{1}{2}D$	25 days	22.34 ±0.52
28.	$L \geq D$	28 days	24.39 ±0.08
29.	$L \geq 1\frac{1}{2}D$	31 days	24.97 ±0.18
30.	$L \geq 2D$	34 days	27.14 ±0.16
31.	Foot Paddle	36 days	27.86 ±0.25
32.	Indentation 4-5	38 days	28.19 ±0.20
33.	Indentation 3-4	40 days	28.79 ±0.09
34.	Indentation 2-3	42 days	30.56 ±0.09
35.	Indentation 1-2	44 days	32.48 ±0.17
36.	Toes 3-5 Separated	46 days	32.51 ±0.21
37.	All Toes Separated	49 days	34.33 ±0.05
38.	Metatarsal tubercles	52 days	35.70 ±0.13
39.	Sub-articular patches	56 days	38.59 ±0.08
40.	Foot Tubercles	61 days	40.89 ±0.15
41.	Fore Limbs Visible	65 days	34.14 ±0.15
42.	Fore Limbs emerge	69 days	33.38 ±0.20
43.	Tail Atrophies	71 days	26.12 ±0.21
44.	Tail Greatly Reduced	72 days	23.68 ±0.14
45.	Tail Stub	73 days	17.57 ±0.89
46.	Metamorphosis Complete	74 days	15.50 ±0.21

Table 30: Age and size of developing *Kaloula pulchra* embryos reared at 16°C – 28°C. (N= Total number of samples examined).

Sl.No.	Stage	Age	Size in mm (N=10)
1.	Fertilization	0 hr	1.48 ±0.43
2.	Gray Crescent	0:25 hr	1.48 ±0.71
3.	2-cell	0:40 hr	1.51 ±0.64
4.	4-cell	1:05 hrs	1.52 ±0.38
5.	8-cell	1:55 hrs	1.50 ±0.27
6.	16-cell	2:50 hrs	1.51 ±0.53
7.	32-cell	5:00 hrs	1.52 ±0.84
8.	Mid Cleavage	6:55 hrs	1.53 ±0.52
9.	Late Cleavage	8:20 hrs	1.55 ±0.27
10.	Dorsal Lip	9:30 hrs	1.55 ±0.63
11.	Yolk Plug	11:15 hrs	1.59 ±1.45
12.	Late Gastrula	12:45 hrs	1.67 ±1.29
13.	Neural Plate	14:55 hrs	1.84 ±0.75
14.	Neural Fold	16:40 hrs	2.39 ±0.63
15.	Rotation	18:05 hrs	2.40 ±1.19
16.	Neural Tube	18:45 hrs	2.47 ±0.42
17.	Tail Bud	19:15 hrs	3.01 ±0.74
18.	Muscular Response	20:10 hrs	3.57 ±0.92
19.	Heart Beat	22:15 hrs	3.90 ±1.23
20.	Tail Elongation (Hatching stage)	1 day 5:30 hrs	4.33 ±1.62
21.	Cornea Transparent	1 day 11 hrs	5.63 ±1.35
22.	Tail Fin Circulation	1 day 21 hrs	5.93 ±1.02
23.	Operculum present	2 days 12 hrs	6.37 ±1.28
24.	Left Gill	2 days 23 hrs	7.24 ±1.83
25.	Spiracles Forms	3 days 15 hrs	9.25 ±2.46
26.	$L < \frac{1}{2}D$	5 days 22 hrs	14.31 ±2.57
27.	$L \geq \frac{1}{2}D$	6 days 18 hrs	18.26 ±1.82
28.	$L \geq D$	8 days	20.49 ±0.70
29.	$L \geq 1\frac{1}{2}D$	10 days	20.51 ±1.03
30.	$L \geq 2D$	12 days	20.54 ±0.97
31.	Foot Paddle	14 days	22.51 ±0.76
32.	Indentation 4-5	16 days	23.64 ±1.56
33.	Indentation 3-4	18 days	24.66 ±0.83
34.	Indentation 2-3	19 days	25.32 ±0.82
35.	Indentation 1-2	21 days	26.49 ±1.65
36.	Toes 3-5 Separated	23 days	27.68 ±0.67
37.	All Toes Separated	28 days	28.27 ±1.29
38.	Metatarsal tubercles	31 days	28.56 ±1.34
39.	Sub-articular patches	33 days	29.42 ±1.53
40.	Foot Tubercles	35 days	29.87 ±1.26
41.	Fore Limbs Visible	38 days	29.64 ±1.72
42.	Fore Limbs Emerge	40 days	28.78 ±0.88
43.	Tail Atrophies	43 days	23.28 ±1.74
44.	Tail Greatly Reduced	45 days	15.53 ±2.04
45.	Tail Stub	46 days	12.16 ±0.64
46.	Metamorphosis Complete	47 days	9.07 ±0.47

Table 31: Age and size of developing *Microhyla berdmorei* embryos reared at 15°C – 25°C. (N= Total number of samples examined).

Sl.No.	Stage	Age	Size in mm (N=10)
1.	Fertilization	0 hr	1.43 ±0.02
2.	Gray Crescent	0. 25 hr	1.43 ±0.04
3.	2-cell	1.05 hr	1.45 ±0.03
4.	4-cell	2:40 hr	1.45 ±0.15
5.	8-cell	4:15 hr	1.47 ±0.04
6.	16-cell	5:25 hr	1.47 ±0.02
7.	32-cell	7:10 hr	1.47 ±0.17
8.	Mid Cleavage	8:55 hr	1.47±0.02
9.	Late Cleavage	10:10 hr	1.49±0.03
10.	Dorsal Lip	11:10 hr	1.66±0.04
11.	Yolk Plug	12:10 hr	1.84±0.05
12.	Late Gastrula	13:40 hr	1.75±0.05
13.	Neural Plate	16:40 hr	1.88±0.04
14.	Neural Fold	18:40 hr	1.96±0.07
15.	Rotation	20:40 hr	2.23±0.04
16.	Neural Tube	22:40 hr	2.35±0.03
17.	Tail Bud	24:10 hr	2.46±0.03
18.	Muscular Response	1 day 1:40 hr	2.66±0.03
19.	Heart Beat (Hatching stage)	1 day 3:40 hr	3.19±0.14
20.	Tail Elongation	1 day 5:40 hr	4.09±0.18
21.	Cornea Transparent	1 day 12:40 hr	5.83±0.08
22.	Tail Fin Circulation	2 days 14 hr	5.88±0.04
23.	Operculum present	3 days 6hr	6.3±0.11
24.	Left Gill	5 days 4hr	6.71±0.05
25.	Spiracles Forms	8 days	10.84±0.41
26.	Limb < ½Diameter	17 days	17.06 ±1.14
27.	Limb ≥ ½Diameter	24 days	17.71 ±1.1
28.	Limb ≥Diameter	42 days	18.4 ±1.08
29.	Limb ≥ 1½Diameter	47 days	18.85 ±0.62
30.	Limb ≥ 2Diameter	52 days	20.97 ±0.33
31.	Foot Paddle	56 days	21.44 ±1.19
32.	Indentation 4-5	59 days	21.44 ±1.37
33.	Indentation 3-4	61 days	23.17 ±0.29
34.	Indentation 2-3	64 days	24.13 ±0.04
35.	Indentation 1-2	67 days	24.31 ±0.62
36.	Toes 3-5 Separated	71 days	24.41 ±0.61
37.	All Toes Separated	74 days	24.53 ±1.77
38.	Metatarsal tubercles	78 days	24.75 ±2.29
39.	Sub-articular patches	82 days	24.82 ±1.94
40.	Foot Tubercles	92 days	24.88 ±1.73
41.	Fore Limbs Visible	99 days	24.73 ±1.47
42.	Fore Limbs emerge	105 days	22.25 ±2.07
43.	Tail Atrophies	106 days	15.41 ±1.35
44.	Tail Greatly Reduced	107 days	13.87 ±0.34
45.	Tail Stub	108 days	10.28 ±0.07
46.	Metamorphosis Complete	109days	8.21 ±0.57

Table 32: Food items of tadpoles of *Euphlyctis cyanophlyctis*

Food items		Operculum Complete (Stage 25)	Hind Limb Bud Development (Stages 26–30)	Toe Development (Stages 31–41)
Bacillariophyceae	<i>Cymbella</i>	+	+	+
	<i>Diatoma</i>	-	+	+
	<i>Navicula</i>	+	+	+
	<i>Pinnularia</i>	+	+	+
	<i>Stauroneis</i>	-	-	+
	<i>Suirirella</i>	-	+	+
	<i>Tabellaria</i>	+	+	+
Chlorophyceae	<i>Closterium</i>	+	+	+
	<i>Cosmarium</i>	+	+	+
	<i>Docidium</i>	+	+	+
	<i>Micrasterias</i>	-	+	+
	<i>Mougeotia</i>	+	+	+
	<i>Oedogonium</i>	+	+	+
	<i>Sirogonium</i>	+	+	+
	<i>Spirogyra</i>	+	+	+
	<i>Pediastrum</i>	+	+	+
	<i>Staurastrum</i>	+	+	+
Cyanophyceae	<i>Anabaena</i>	-	+	+
	<i>Aphanothecce</i>	-	+	+
	<i>Nostoc</i>	-	+	+
	<i>Oscillatoria</i>	+	+	+
	<i>Pandorina</i>	-	+	+
	<i>Spirulina</i>	-	+	+
Cryptophyceae	<i>Arcella</i>	+	+	+
	<i>Cryptomonas</i>	+	+	+
Euglenophyceae	<i>Euglena</i>	-	+	+
	<i>Phacus</i>	+	+	+
Zooplanktons	<i>Brachionus</i>	-	+	+
	<i>Chydorus</i>	-	+	+
	<i>Diffugia</i>	-	-	+
	<i>Euglypha</i>	-	+	+
	<i>Lecane</i>	-	+	+
	<i>Paramecium</i>	-	-	+

+ = Occurrence

- = Non occurrence

Table 33: Food items of tadpoles of *Hylarana nicobariensis*

Food items		Operculum Complete (Stage 25)	Hind Limb Bud Development (Stages 26–30)	Toe Development (Stages 31–41)
Bacillariophyceae	<i>Diatoma</i>	-	+	+
	<i>Navicula</i>	+	+	+
	<i>Stauroneis</i>	+	+	+
	<i>Tabellaria</i>	+	+	+
Chlorophyceae	<i>Cladophora</i>	+	+	+
	<i>Closterium</i>	-	+	+
	<i>Cosmarium</i>	+	+	+
	<i>Docidium</i>	+	+	+
	<i>Mougeotia</i>	+	+	+
	<i>Oedogonium</i>	+	+	+
	<i>Sirogonium</i>	+	+	+
	<i>Spirogyra</i>	+	+	+
	<i>Pediastrum</i>	+	+	+
	<i>Staurastrum</i>	+	+	+
Cyanophyceae	<i>Anabaena</i>	-	+	+
	<i>Nostoc</i>	-	+	+
	<i>Oscillatoria</i>	+	+	+
Cryptophyceae	<i>Arcella</i>	+	+	+
	<i>Cryptomonas</i>	+	+	+
Euglenophyceae	<i>Euglena</i>	-	+	+
	<i>Phacus</i>	-	+	+
Zooplanktons	<i>Brachionus</i>	-	-	+
	<i>Centropyxis</i>	-	-	+
	<i>Chydorus</i>	-	-	+
	<i>Diffugia</i>	-	-	+
	<i>Lecane</i>	-	+	+
	<i>Paramecium</i>	-	-	+

+ = Occurrence

- = Non occurrence

Table 34: Food items of tadpoles of *Kaloula pulchra*

Food items		Operculum Complete (Stage 25)	Hind Limb Bud Development (Stages 26–30)	Toe Development (Stages 31–41)
Bacillariophyceae	<i>Diatoma</i>	+	+	+
	<i>Navicula</i>	+	+	+
	<i>Pinnularia</i>	-	+	+
	<i>Stauroneis</i>	+	+	+
	<i>Tabellaria</i>	+	+	+
Chlorophyceae	<i>Closterium</i>	-	+	+
	<i>Cosmarium</i>	+	+	+
	<i>Crucigenia</i>	+	+	+
	<i>Snedesmus</i>	+	+	+
	<i>Spirogyra</i>	+	+	+
Cyanophyceae	<i>Anabaena</i>	+	+	+
	<i>Nostoc</i>	+	+	+
	<i>Oscillatoria</i>	+	+	+
Cryptophyceae	<i>Arcella</i>	+	+	+
	<i>Cryptomonas</i>	+	+	+
Euglenophyceae	<i>Euglena</i>	-	+	+
	<i>Phacus</i>	-	-	+
Zooplanktons	<i>Lecane</i>	-	+	+
	<i>Paramecium</i>	-	-	+

+ = Occurrence

- = Non occurrence

Table 35: Food items of tadpoles of *Microhyla berdmorei*

Food items		Operculum Complete (Stage 25)	Hind Limb Bud Development (Stages 26–30)	Toe Development (Stages 31–41)
Bacillariophyceae	<i>Cymbella</i>	-	+	+
	<i>Diatoma</i>	+	+	+
	<i>Fragilaria</i>	+	+	+
	<i>Melosira</i>	-	+	+
	<i>Navicula</i>	+	+	+
	<i>Pinnularia</i>	-	+	+
	<i>Stauroneis</i>	+	+	+
	<i>Synedra</i>	+	+	+
	<i>Tabellaria</i>	+	+	+
Chlorophyceae	<i>Cladophora</i>	+	+	+
	<i>Closterium</i>	+	+	+
	<i>Cosmarium</i>	+	+	+
	<i>Docidium</i>	-	-	+
	<i>Mougeotia</i>	-	+	+
	<i>Oedogonium</i>	-	+	+
	<i>Sirogonium</i>	-	+	+
	<i>Spirogyra</i>	+	+	+
	<i>Ulothrix</i>	+	+	+
	<i>Staurastrum</i>	+	+	+
Cyanophyceae	<i>Anabaena</i>	-	+	+
	<i>Nostoc</i>	-	+	+
	<i>Oscillatoria</i>	+	+	+
Cryptophyceae	<i>Arcella</i>	+	+	+
	<i>Cryptomonas</i>	+	+	+
Euglenophyceae	<i>Euglena</i>	-	+	+
	<i>Phacus</i>	-	-	+
Zooplanktons	<i>Centropyxis</i>	-	-	+
	<i>Euglypha</i>	-	+	+
	<i>Lecane</i>	+	+	+
	<i>Paramecium</i>	-	-	+

+ = Occurrence

- = Non occurrence

Table 36(a): Length of intestine of *Euphlyctis cyanophlyctis* at different developmental stages:

Gosner Stage	Total length (in mm)	Gut length (in mm)	Gosner Stage	Total length (in mm)	Gut length (in mm)
25	15.20	58.98	36	46.25	208.72
26	21.65	64.72	37	48.14	237.95
27	24.43	74.78	38	54.35	243.0
28	26.41	86.65	39	58.34	255.54
29	28.85	88.05	40	65.86	337.24
30	31.08	95.98	41	64.28	182.55
31	31.43	125.65	42	60.05	124.56
32	32.87	147.42	43	52.76	76.54
33	35.15	164.23	44	38.31	40.84
34	37.92	182.22	45	32.56	40.56
35	42.53	198.87	46	19.89	48.42

Table 36(b): Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Euphlyctis cyanophlyctis*.

Correlations				
		Stage	Total length	Gut length
Pearson Correlation	Stage	1	0.978**	0.976**
	Total length	0.978**	1	0.977**
	Gut length	0.976**	0.978**	1
Sig. (2-tailed)	Stage	.	0.000	0.000
	Total length	0.000	.	0.000
	Gut length	0.000	0.000	.
N	Stage	16	16	16
	Total length	16	16	16
	Gut length	16	16	16

** Correlation is significant at the 0.01 level (2-tailed).

Table 36(c): Correlations between developmental stages from stage 40 to stage 46 along with total lengths and gut lengths of *Euphlyctis cyanophlyctis*.

Correlations				
		Stage	Total length	Gut length
Pearson Correlation	Stage	1	-0.974**	-0.877**
	Total length	-0.974**	1	0.761*
	Gut length	-0.877**	0.761*	1
Sig. (2-tailed)	Stage	.	0.000	0.009
	Total length	0.000	.	0.0470
	Gut length	0.009	0.047	.
N	Stage	7	7	7
	Total length	7	7	7
	Gut length	7	7	7

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed).

Table 37(a): Length of intestine of *Hylarana nicobariensis* at different developmental stages

Gosner Stage	Total length (in mm)	Gut length (in mm)	Gosner Stage	Total length (in mm)	Gut length (in mm)
25	13.67	22.04	36	32.78	65.22
26	18.82	28.65	37	34.23	66.87
27	21.65	34.62	38	36.24	68.20
28	24.12	40.23	39	38.76	69.82
29	25.80	44.08	40	40.94	74.56
30	26.64	47.45	41	34.06	68.64
31	27.52	48.75	42	33.12	52.31
32	28.06	56.85	43	26.52	38.65
33	29.21	61.86	44	23.61	26.42
34	30.87	62.06	45	17.86	18.59
35	32.68	64.31	46	15.83	12.80

Table 37(b): Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Hylarana nicobariensis*.

Correlations				
		Stage	Total length	Gut length
Pearson Correlation	Stage	1	0.978**	0.972**
	Total length	0.978**	1	0.971**
	Gut length	0.972**	0.971**	1
Sig. (2-tailed)	Stage	.	0.000	0.000
	Total length	0.000	.	0.000
	Gut length	0.000	0.000	.
N	Stage	16	16	16
	Total length	16	16	16
	Gut length	16	16	16

**Correlation is significant at the 0.01 level (2-tailed).

Table 37(c): Correlations between developmental stages from stage 40 to stage 46 along with total lengths and gut lengths of *Hylarana nicobariensis*.

Correlations				
		Stage	Total length	Gut length
Pearson Correlation	Stage	1	-0.990**	-0.990**
	Total length	-0.990**	1	0.978*
	Gut length	-0.990**	0.978*	1
Sig. (2-tailed)	Stage	.	0.000	0.000
	Total length	0.000	.	0.000
	Gut length	0.000	0.000	.
N	Stage	7	7	7
	Total length	7	7	7
	Gut length	7	7	7

**Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed).

Table 38(a): Length of intestine of *Kaloula pulchra* at different developmental stages

Gosner Stage	Total length (in mm)	Gut length (in mm)	Gosner Stage	Total length (in mm)	Gut length (in mm)
25	9.56	21.64	36	28.24	37.45
26	14.04	24.23	37	28.37	42.80
27	17.83	25.52	38	29.21	54.32
28	20.85	26.48	39	29.40	57.85
29	21.26	27.42	40	29.92	62.75
30	21.30	28.36	41	29.78	46.72
31	22.24	29.84	42	28.85	30.52
32	24.12	31.25	43	23.36	29.87
33	25.20	32.91	44	15.76	21.45
34	25.51	34.27	45	12.04	20.26
35	26.28	35.65	46	9.12	12.82

Table 38(b): Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Kaloula pulchra*.

Correlations					
		Stage	Total length	Gut length	
Pearson Correlation	Stage	1	0.945**	0.921**	
	Total length	0.945**	1	0.805**	
	Gut length	0.921**	0.805**	1	
Sig. (2-tailed)	Stage	.	0.000	0.000	
	Total length	0.000	.	0.000	
	Gut length	0.000	0.000	.	
N	Stage	16	16	16	
	Total length	16	16	16	
	Gut length	16	16	16	

**Correlation is significant at the 0.01 level (2-tailed).

Table 38(c): Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Kaloula pulchra*.

Correlations					
		Stage	Total length	Gut length	
Pearson Correlation	Stage	1	-0.965**	-0.947**	
	Total length	-0.965**	1	0.844*	
	Gut length	-0.947**	0.844*	1	
Sig. (2-tailed)	Stage	.	0.000	0.001	
	Total length	0.000	.	0.017	
	Gut length	0.001	0.017	.	
N	Stage	7	7	7	
	Total length	7	7	7	
	Gut length	7	7	7	

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

Table 39(a): Length of intestine of *Microhyla berdmorei* at different developmental stages:

Gosner Stage	Total length (in mm)	Gut length (in mm)	Gosner Stage	Total length (in mm)	Gut length (in mm)
25	11.24	12.20	36	24.61	43.18
26	16.78	15.64	37	24.68	44.20
27	17.34	18.80	38	24.80	45.63
28	18.40	21.55	39	24.85	47.86
29	18.96	23.04	40	25.24	48.53
30	21.20	26.87	41	24.43	24.80
31	21.46	29.02	42	22.08	21.27
32	21.75	33.90	43	15.25	17.82
33	22.87	34.32	44	14.10	14.26
34	23.63	37.80	45	10.62	11.24
35	24.42	41.67	46	8.21	8.68

Table 39(b): Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Microhyla berdmorei*.

Correlations					
		Stage	Total length	Gut length	
Pearson Correlation	Stage	1	0.921**	0.992**	
	Total length	0.921**	1	0.947**	
	Gut length	0.992**	0.947**	1	
Sig. (2-tailed)	Stage	.	0.000	0.000	
	Total length	0.000	.	0.000	
	Gut length	0.000	0.000	.	
N	Stage	16	16	16	
	Total length	16	16	16	
	Gut length	16	16	16	

**Correlation is significant at the 0.01 level (2-tailed).

Table 39(c): Correlations between developmental stages from stage 40 to stage 46 along with total lengths and gut lengths of *Microhyla berdmorei*.

Correlations					
		Stage	Total length	Gut length	
Pearson Correlation	Stage	1	-0.982**	-0.886**	
	Total length	-0.982**	1	0.827*	
	Gut length	-0.886**	0.827*	1	
Sig. (2-tailed)	Stage	.	0.000	0.008	
	Total length	0.000	.	0.022	
	Gut length	0.008	0.022	.	
N	Stage	7	7	7	
	Total length	7	7	7	
	Gut length	7	7	7	

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

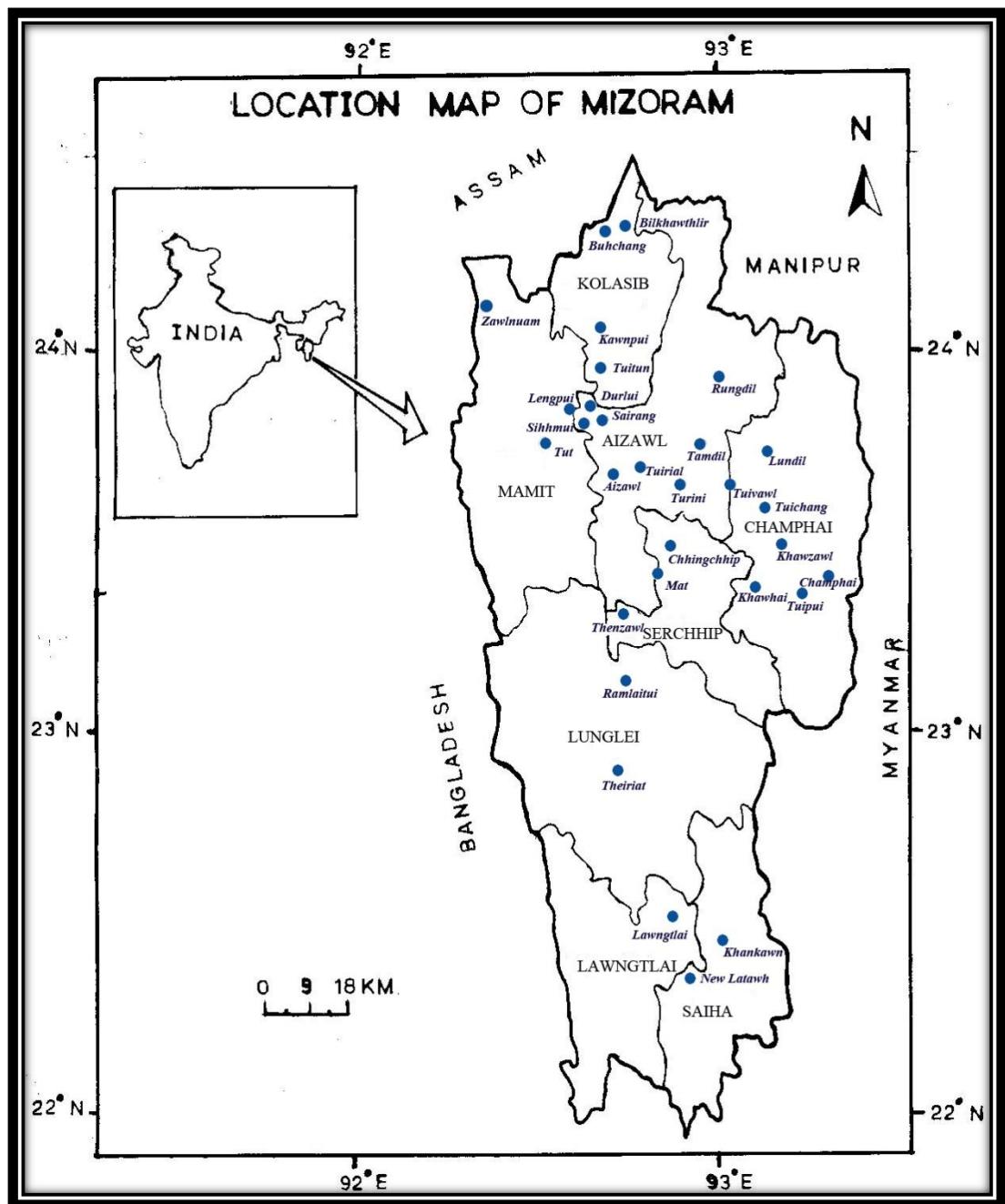


Fig. 4.1: Map of Mizoram showing the surveyed areas.

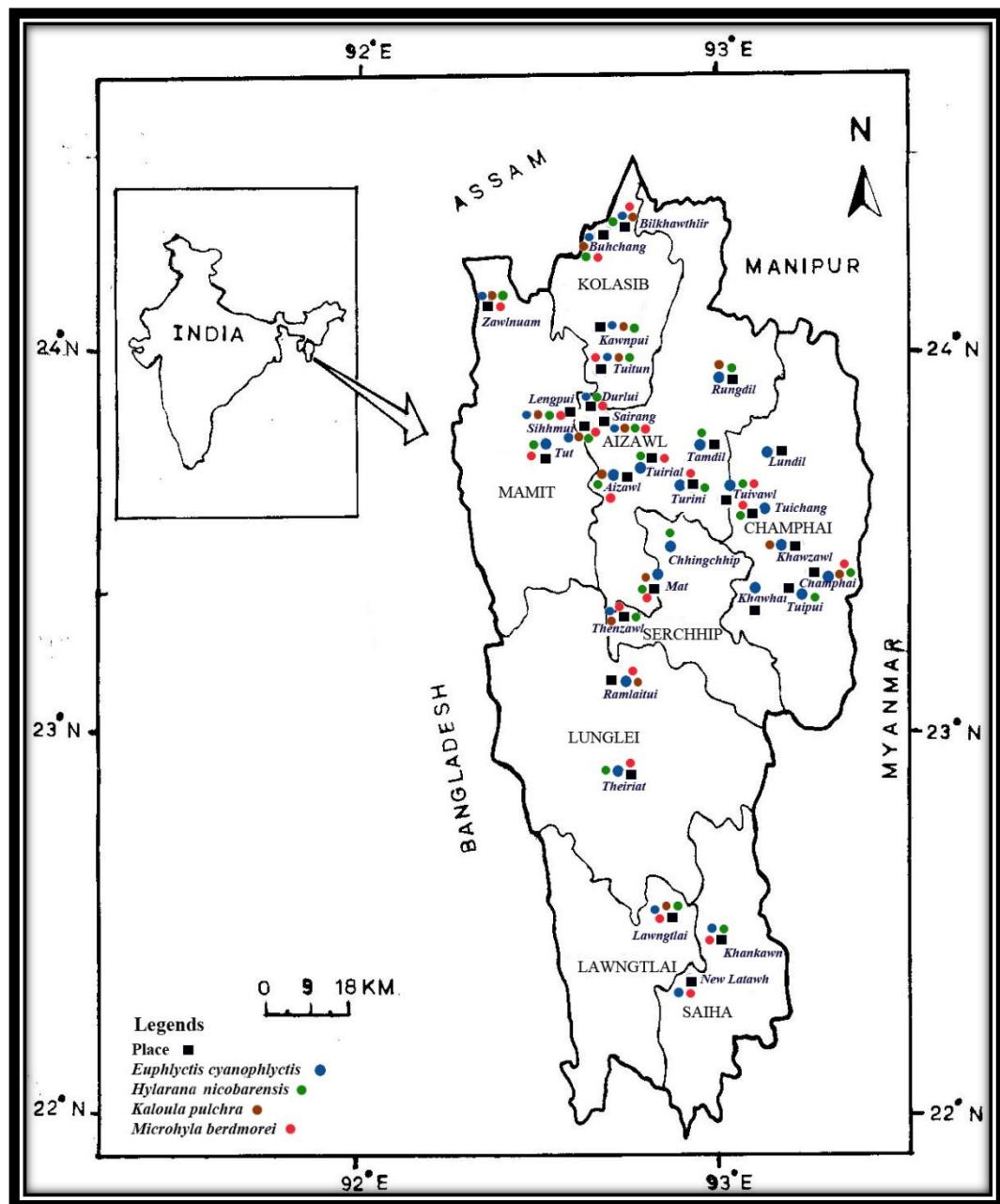


Fig. 4.2: Map of Mizoram showing the distribution of ranids and microhylids prevalent in the state.



Fig. 4.3 (a & b): *Euphlyctis cyanophlyctis* (a.) Male with vocal slit, and (b.) Female

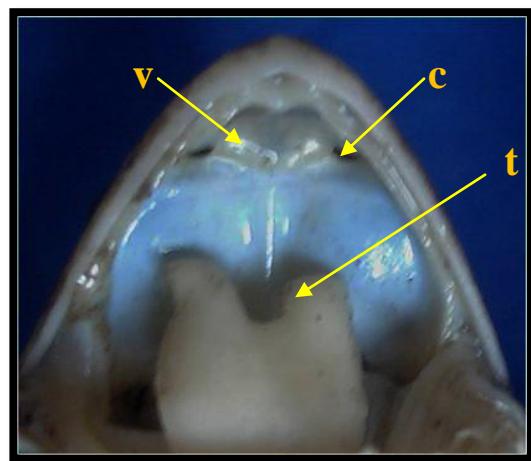


Fig. 4.4: Mouth of *Euphlyctis cyanophlyctis* showing bifid tongue (t), chaonae (c) and vomerine teeth (v).

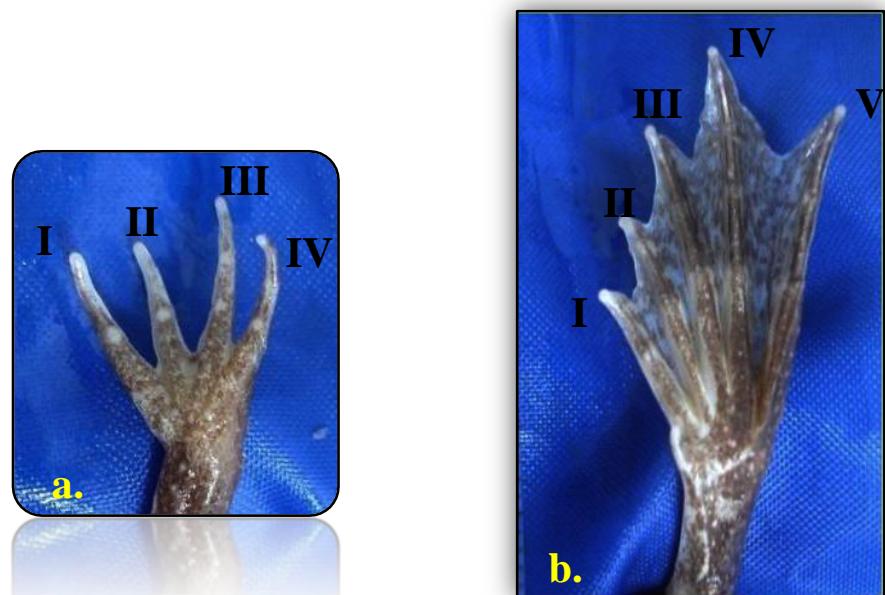


Fig. 4.5 (a & b): Left hand and left foot of *Euphlyctis cyanophlyctis*
(Webbing formula: I₀₋₀II₀₋₀III₀₋₀IV₀₋₀V).



Fig. 4.6: The breeding ground of *Euphlyctis cyanophlyctis* at Study site-I, Kawnpu



Fig. 4.7: The breeding ground of *Euphlyctis cyanophlyctis* at Study site-IV, Tlawng river

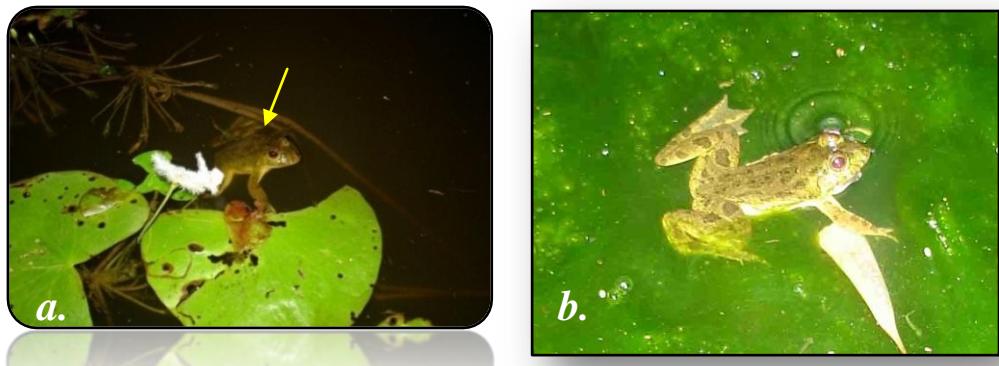


Fig. 4.8 (a&b): Male *Euphlyctis cyanophlyctis* calls while floating on the water surface.

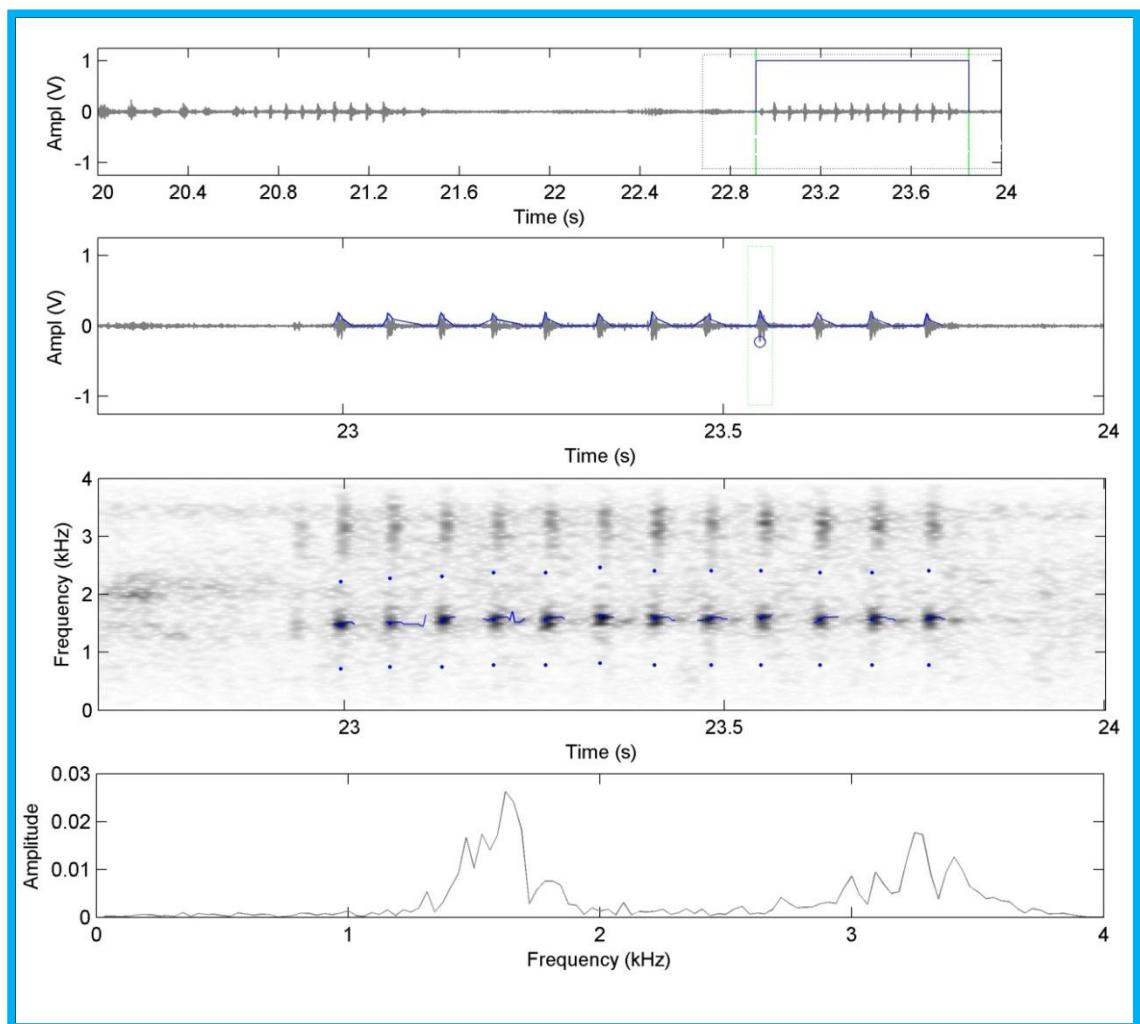


Fig. 4.9: Oscillogram, sonogram and frequency spectrum of an advertisement call of *Euphlyctis cyanophlyctis*

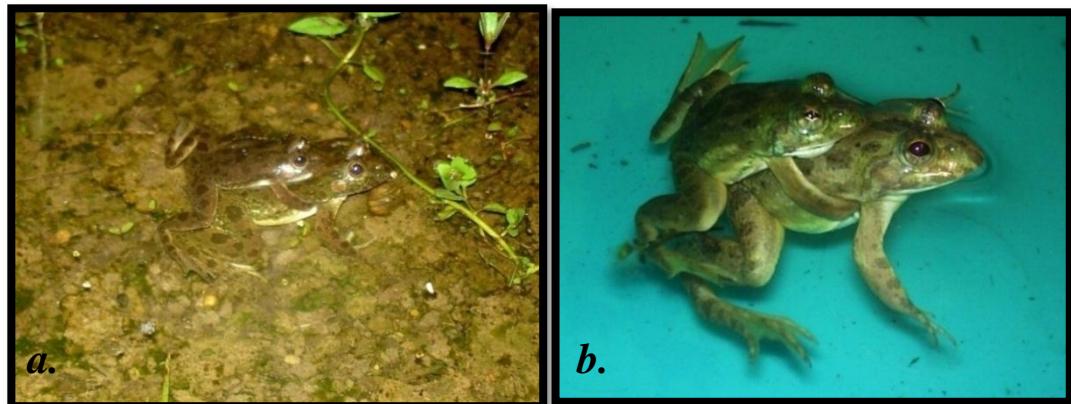


Fig. 4.10 (a & b): Amplexing pairs of *Euphlyctis cyanophlyctis* (a.) in the natural environment, and (b.) in the laboratory condition.



Fig. 4.11: Amplexing pairs of *Euphlyctis cyanophlyctis*, after laying eggs in the terrarium.



Fig. 4.12: Eggs of *Euphlyctis cyanophlyctis* in the natural environment.

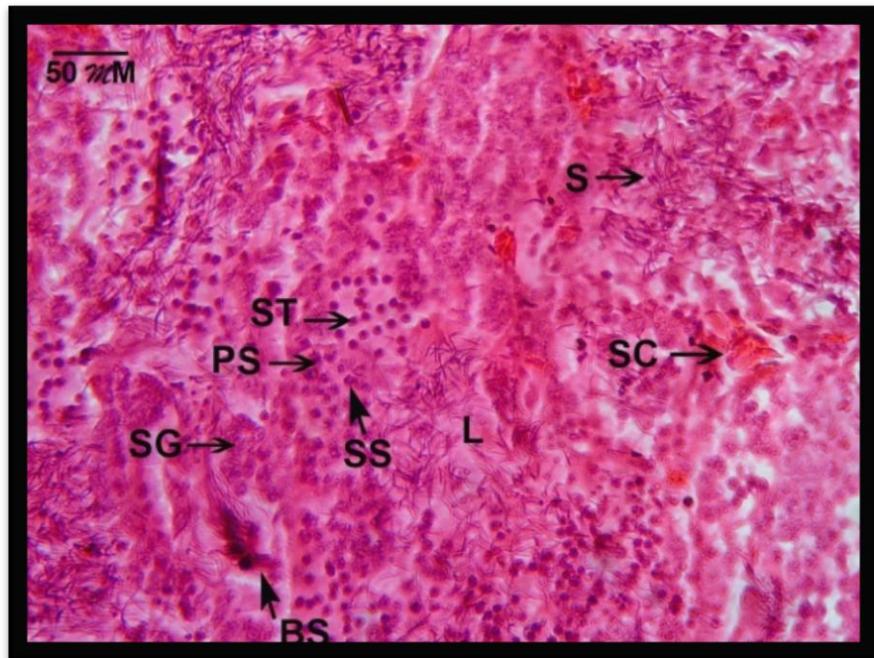


Fig. 4.13: Section of testis of *Euphylyctis cyanophlyctis* during the breeding season. Spermatogonia (SG), Primary spermatocytes (PS), Secondary spermatocytes (SS), Spermatids (ST), Spermatozoa (S), Bundles of spermatozoa (BS), Lumen of seminiferous tubules (L) and Sertoli cells (SC).

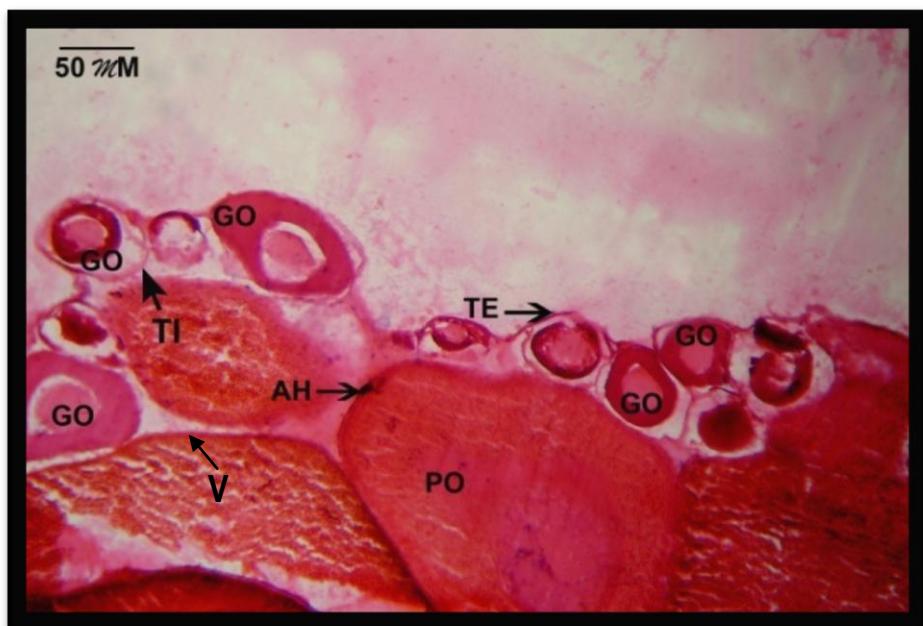


Fig. 4.14: Section of ovary of *Euphylyctis cyanophlyctis* during the breeding season. Primary oocytes (PO), Growing oocytes (GO), Animal hemisphere (AH), Vitelline membrane (V), Theca externa (TE) and Theca interna (TI).

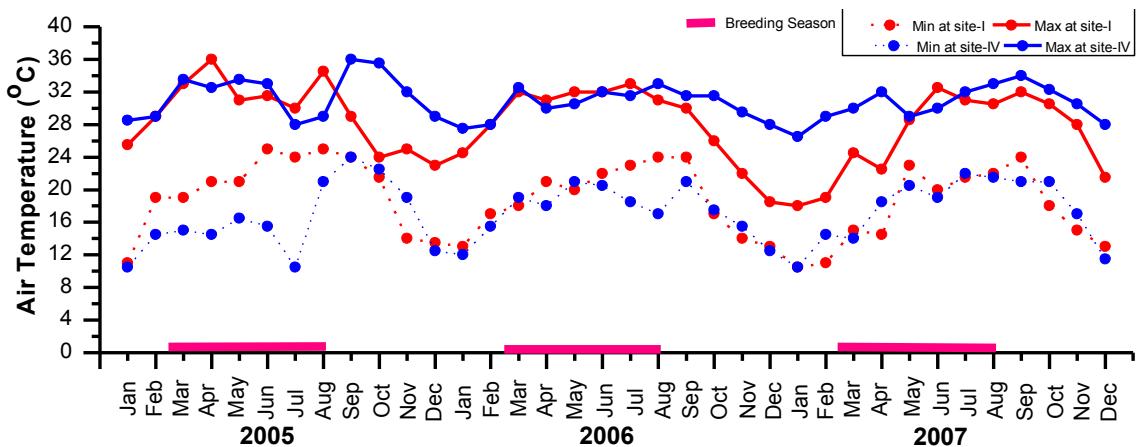


Fig. 4.15: Air temperature in the study sites I and IV from 2005 to 2007

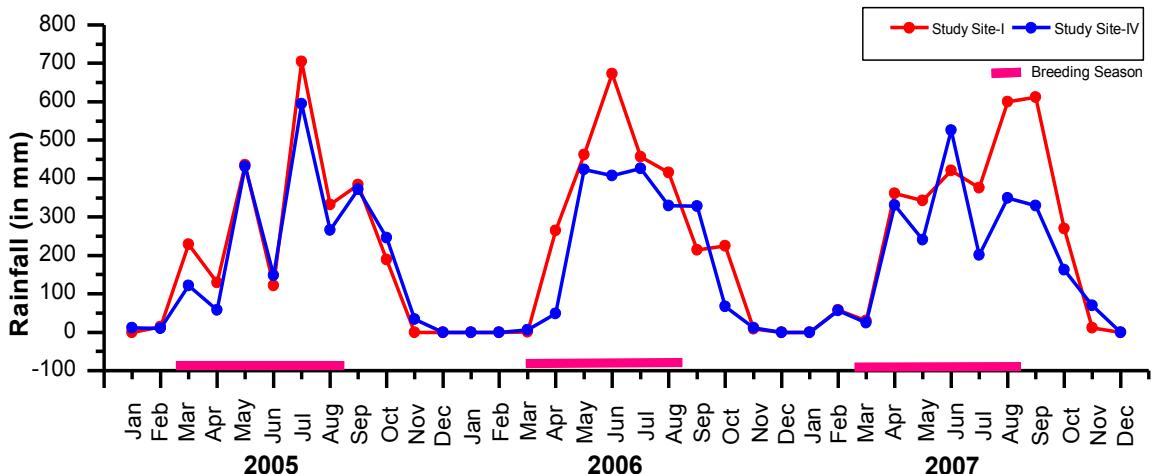


Fig. 4.16: Rainfall in the study sites I and IV from 2005 to 2007

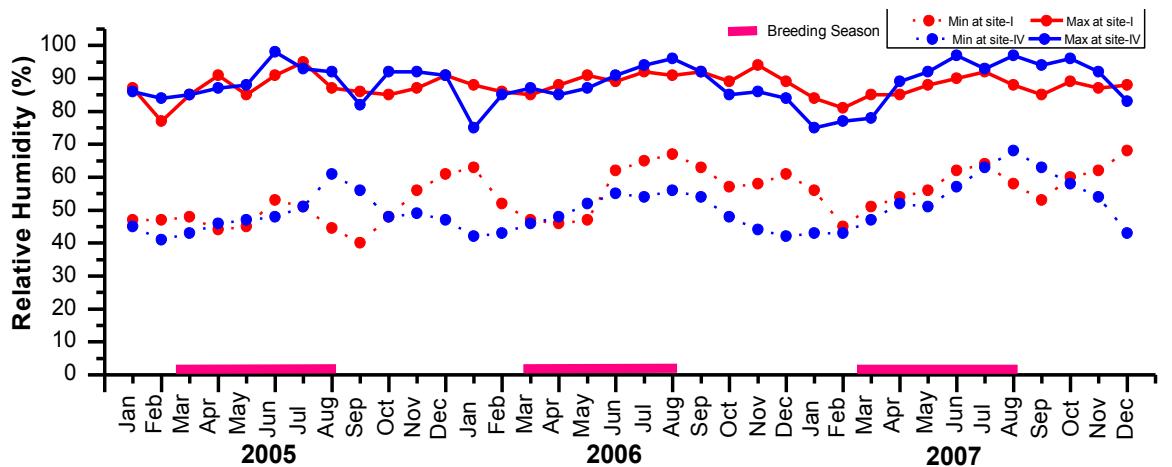


Fig. 4.17: Relative humidity in the study sites I and IV from 2005 to 2007

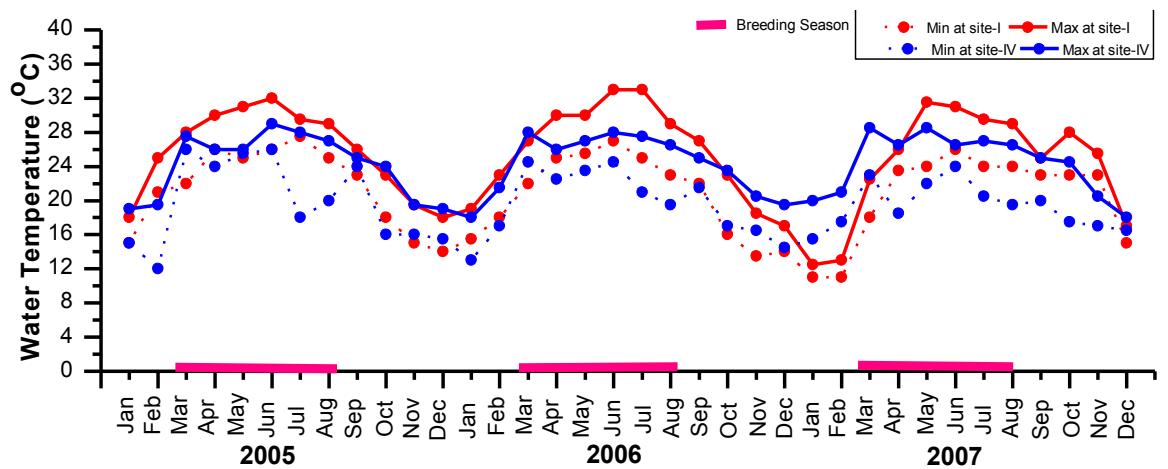


Fig. 4.18: Water temperature in the study sites I and IV from 2005 to 2007

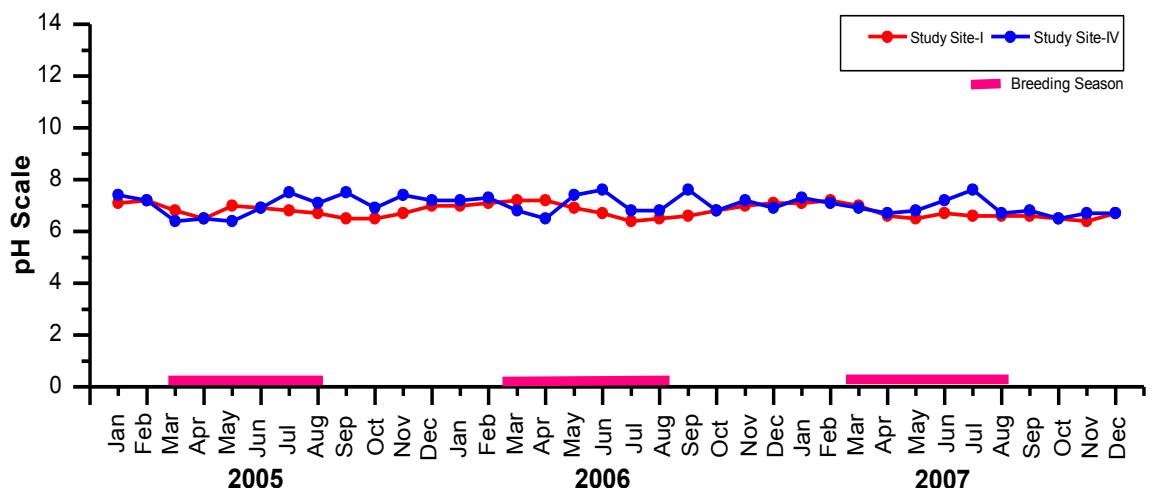


Fig. 4.19: pH of water in the study sites I and IV from 2005 to 2007



Fig. 4.20 (a&b): *Hylarana nicobariensis* (a.) Male and (b.) Female

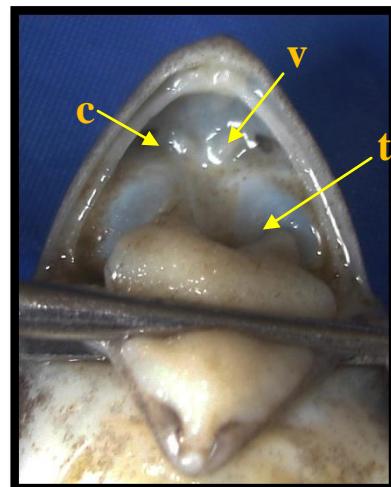


Fig. 4.21: Mouth of *Hylarana nicobariensis* showing bifid tongue (t), chaonae (c) and vomerine teeth (v)

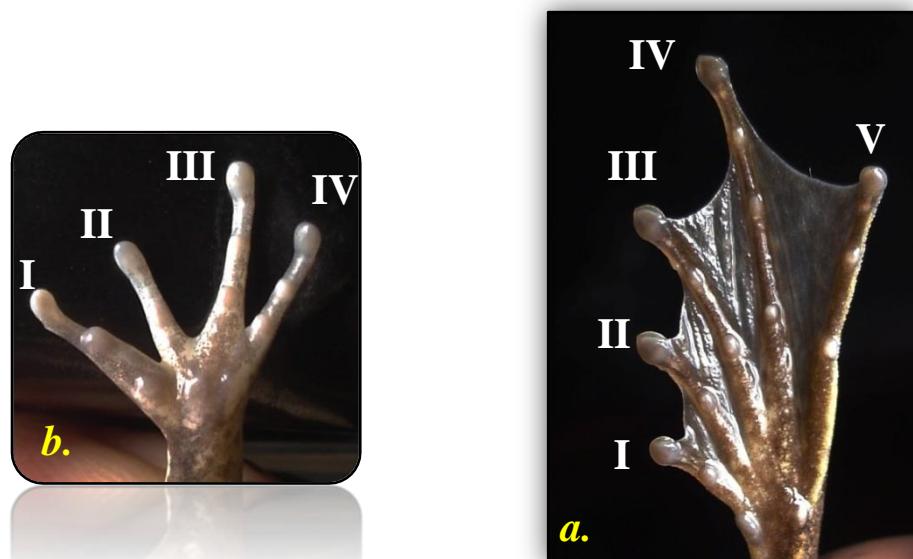


Fig. 4.22 (a&b): Left hand and left foot of *Hylarana nicobariensis*
(Webbing formula: I_{1½}II₁₋₂III₁₋₂IV_{1½-1}V)



Fig. 4.23: Study site IV (Tlawng river) during the non-breeding season.

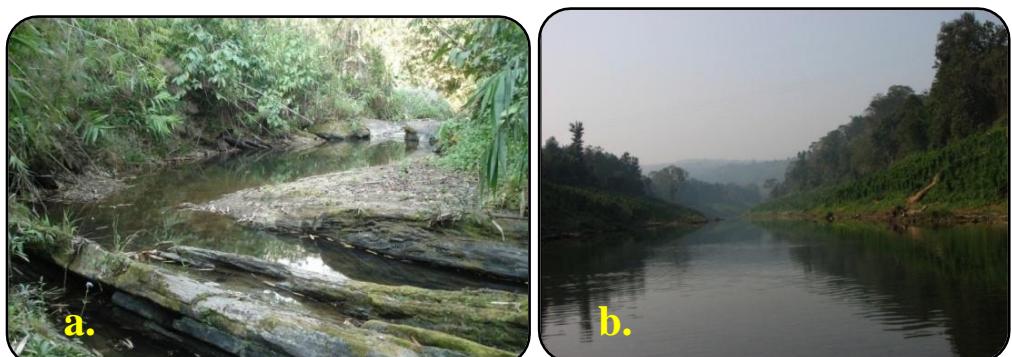


Fig. 4.24 (a&b): The breeding sites of *Hylarana nicobariensis* (a.) at study site II (Tuitun stream), and (b.) at study site IV (Tlawng river)

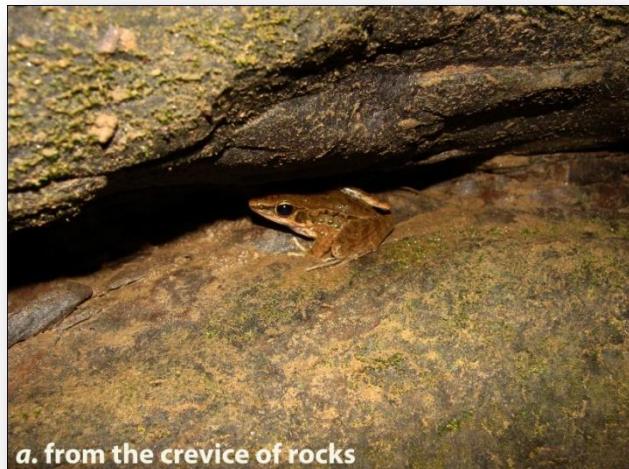


Fig. 4.25 (a-e): Different calling sites of male *Hylarana nicobariensis*.

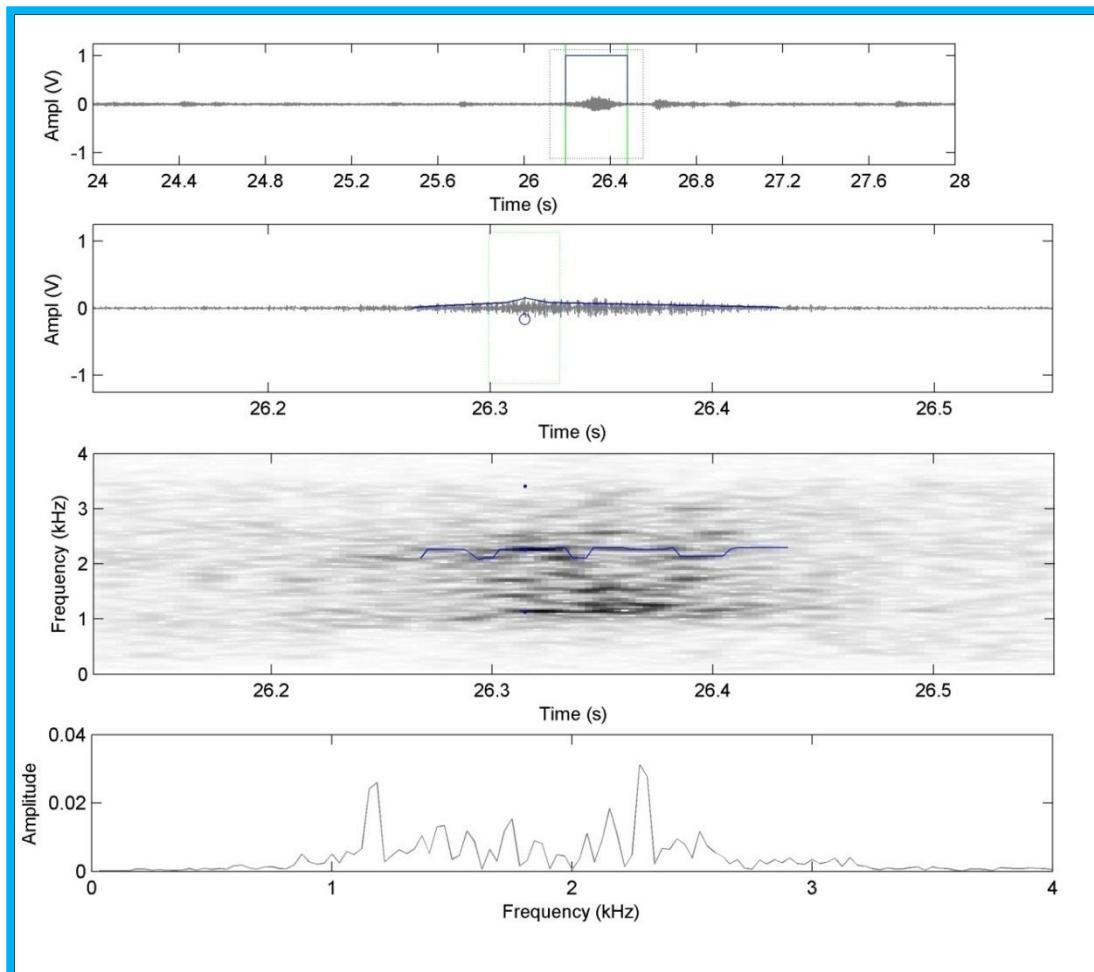


Fig. 4.26: Oscillogram, sonogram and frequency spectrum of an advertisement call of *Hylarana nicobariensis*



Fig. 4.27: Gravid female of *Hylarana nicobariensis* approaching the breeding ground.



Fig. 4.28 (a–e): Different amplexing sites for *Hylarana nicobariensis*



Fig. 4.29 (a&b): Male and female *Hylarana nicobariensis*, after laying eggs (a.) in the natural environment, and (b.) in the laboratory condition.



Fig. 4.30: Group of egg clutches of *Hylarana nicobariensis* in the natural environment.



Fig. 4.31(a-c): Different oviposition sites of *Hylarana nicobariensis* in the study sites.

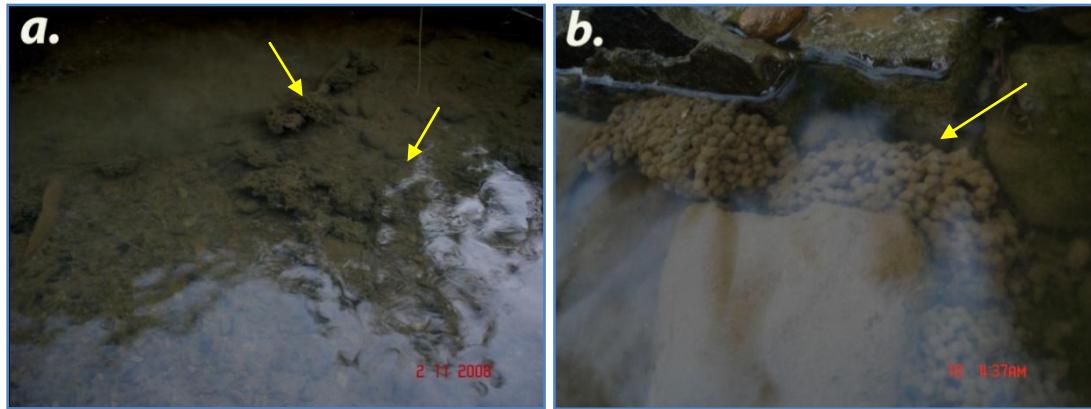


Fig. 4.32 (a&b): Communal egg masses deposited by females of *Hylarana nicobariensis*

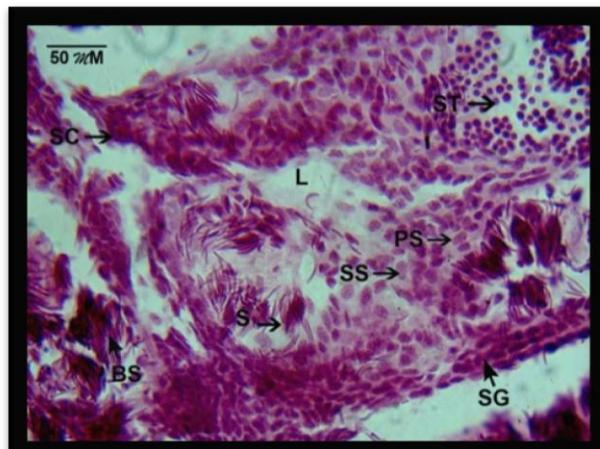


Fig. 4.33: Section of testis of *Hylarana nicobariensis* during the breeding season. Spermatogonia (SG), Primary spermatocytes (PS), Secondary spermatocytes (SS), Spermatids (ST), Spermatozoa (S), Bundles of spermatozoa (BS), Lumen of seminiferous tubules (L) and Sertoli cells (SC).

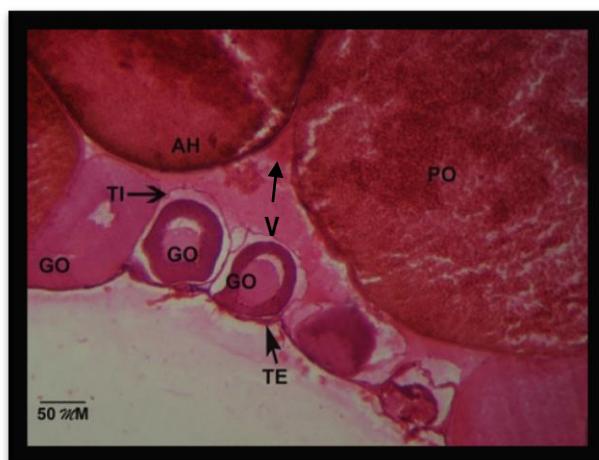


Fig. 4.34: Section of ovary of *Hylarana nicobariensis* during the breeding season. Primary oocytes (PO), Growing oocytes (GO), Animal hemisphere (AH), Vitelline membrane, Theca externa (TE) and Theca interna (TI).

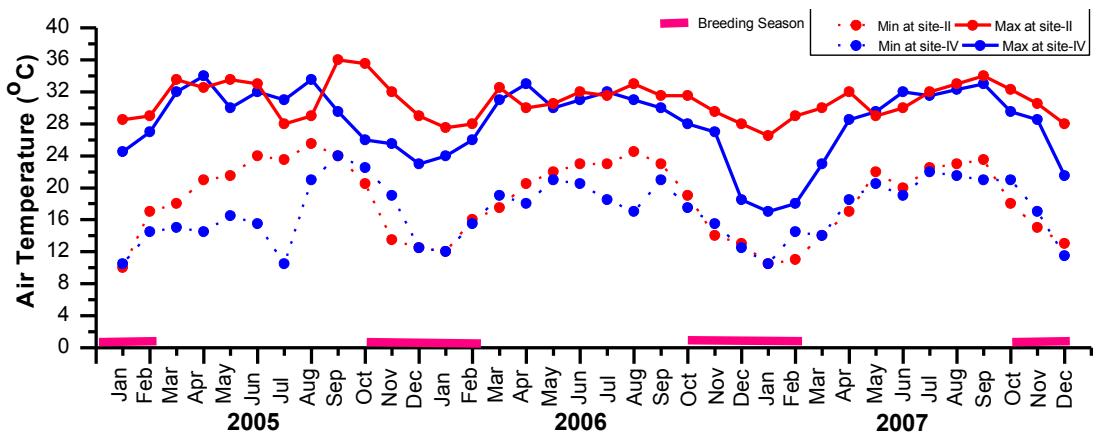


Fig. 4.35: Air temperature in the study sites II and IV from 2005 to 2007

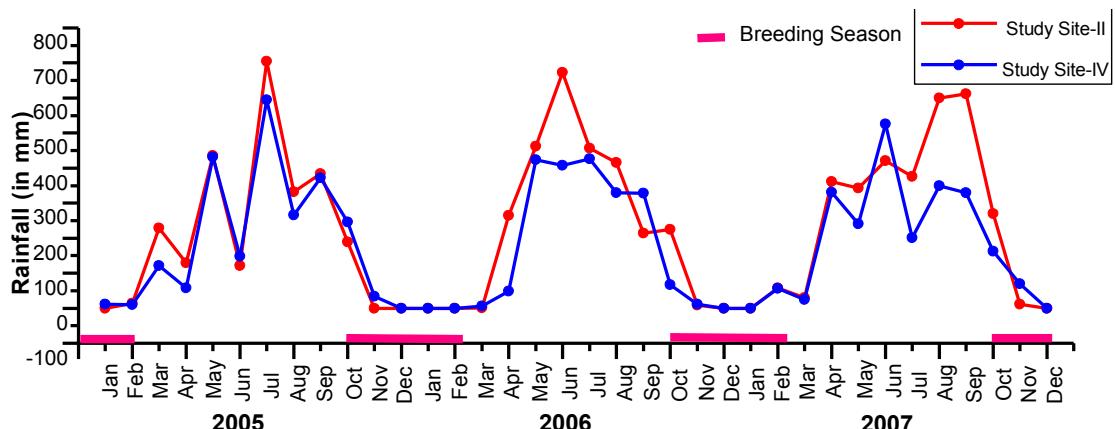


Fig. 4.36: Rainfall in the study sites II and IV from 2005 to 2007

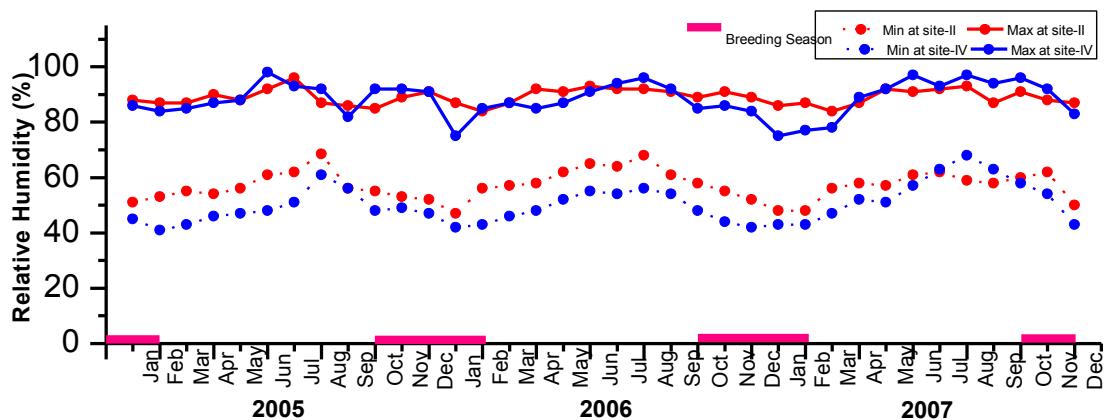


Fig. 4.37: Relative humidity in the study sites II and IV from 2005 to 2007

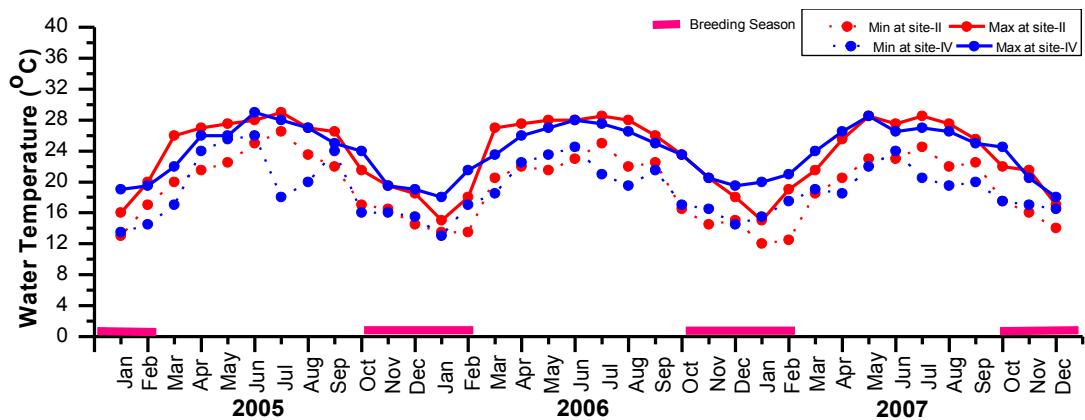


Fig. 4.38: Water temperature in the study sites II and IV from 2005 to 2007

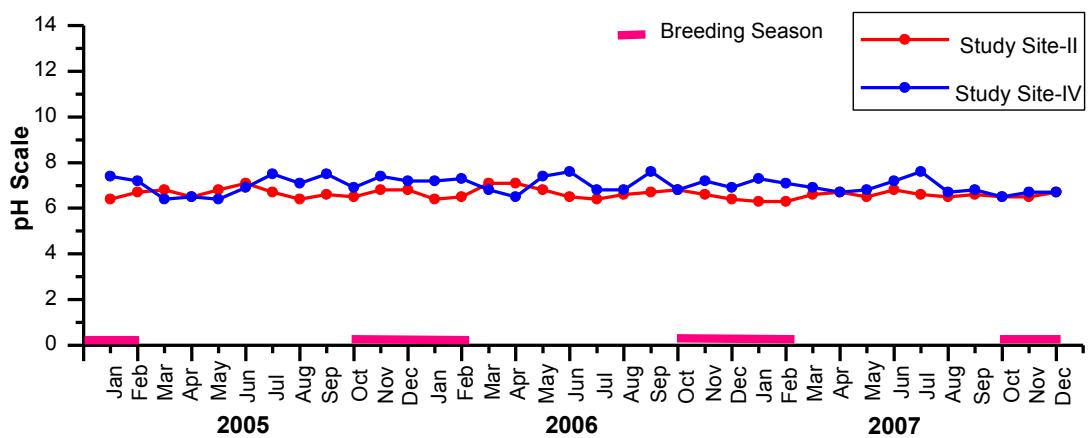


Fig. 4.39: pH of water in the study sites II and IV from 2005 to 2007



Fig. 4.40: Male and female *Kaloula pulchra*

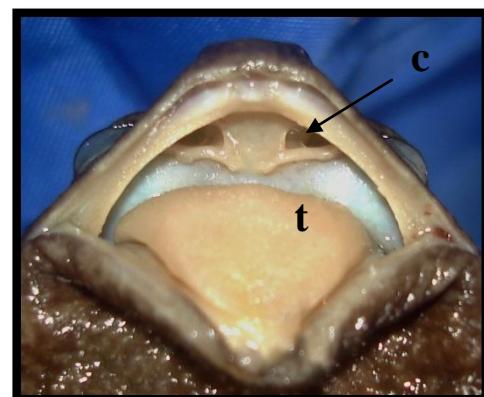


Fig. 4.41: Mouth of *Kaloula pulchra*. t: tongue; c: chaonae.

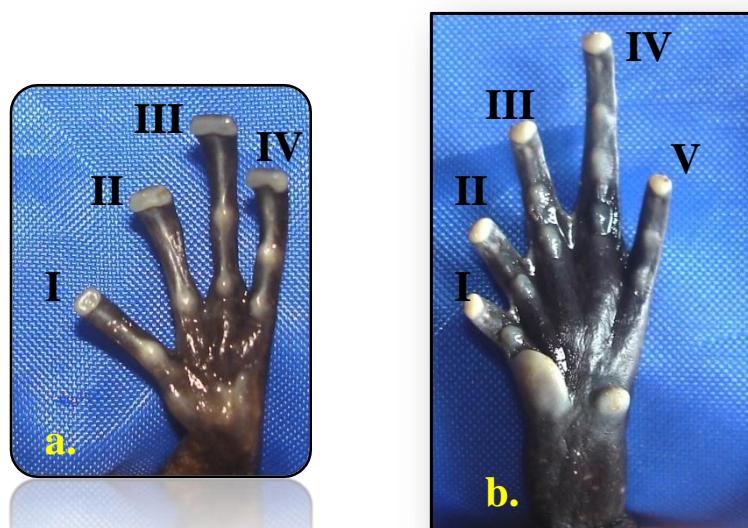


Fig. 4.42 (a&b): Left hand and left foot of *Kaloula pulchra*



Fig. 4.43(a&b): Rock-pools in the breeding sites of *Kaloula pulchra* at Study site-III

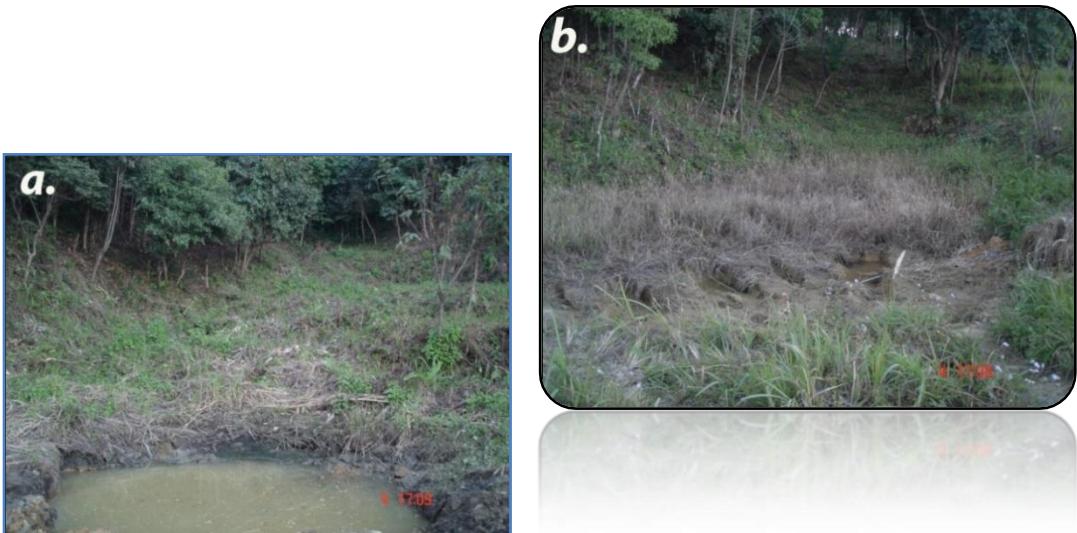


Fig. 4.44 (a&b): Temporary pools, the breeding sites of *Kaloula pulchra* at Study site-V



Fig. 4.45 (a&b): Male *Kaloula pulchra* calling from the breeding ground

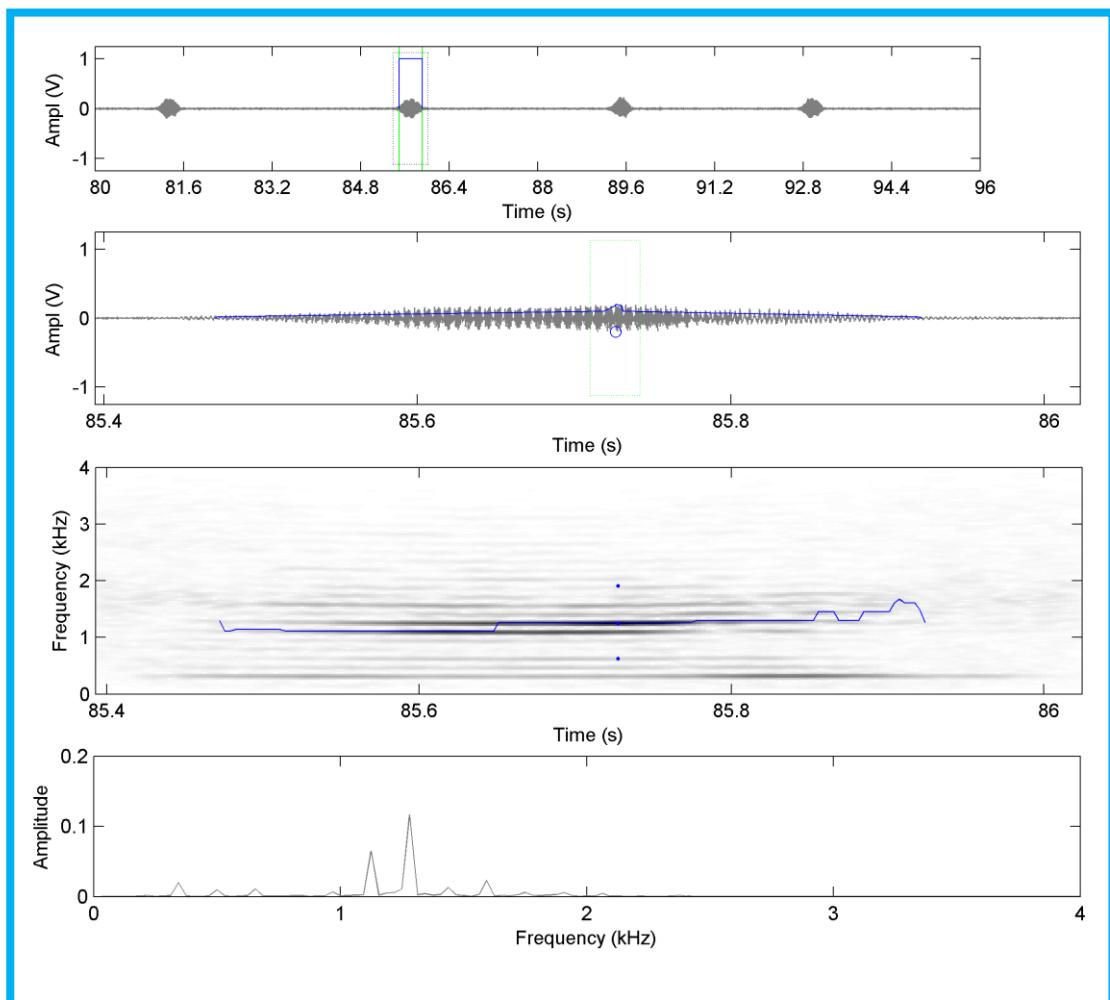


Fig. 4.46: Oscillogram, sonogram and frequency spectrum of an advertisement call of *Kaloula pulchra*

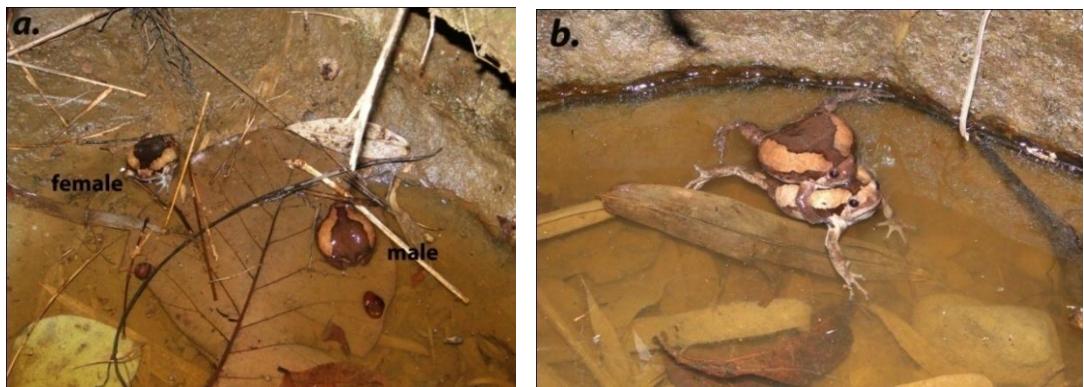


Fig. 4.47 (a&b): Mating behavior of *Kaloula pulchra* (a.) Female enters the breeding ground and approaching the calling male; (b.) Male immediately grabs the female from her back



Fig. 4.48: A dominant male *Kaloula pulchra* amplexes with the female while other male continues to call

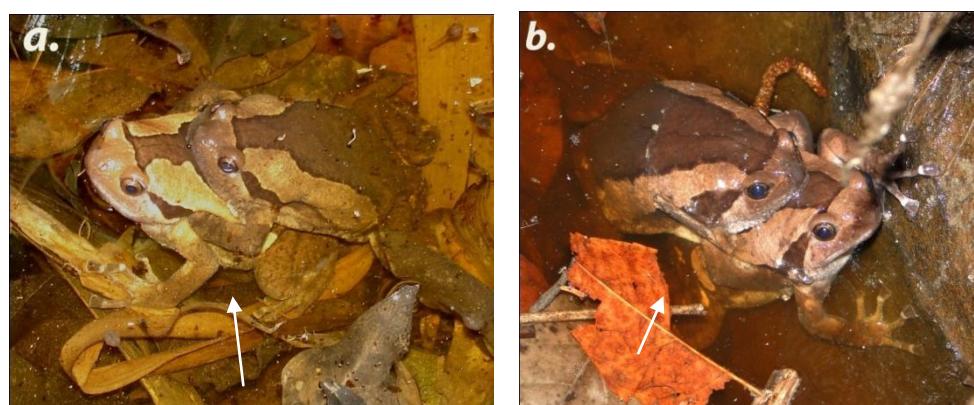


Fig. 4.49 (a&b): Axillary amplexing in *Kaloula pulchra*



Fig. 4.50: Amplexing pair of *Kaloula pulchra*, after laying eggs.



Fig. 4.51: After laying eggs, female *Kaloula pulchra* left the breeding ground, later followed by male.



Fig. 4.52 (a&b): Eggs were laid on the water surface mixed with leave debris

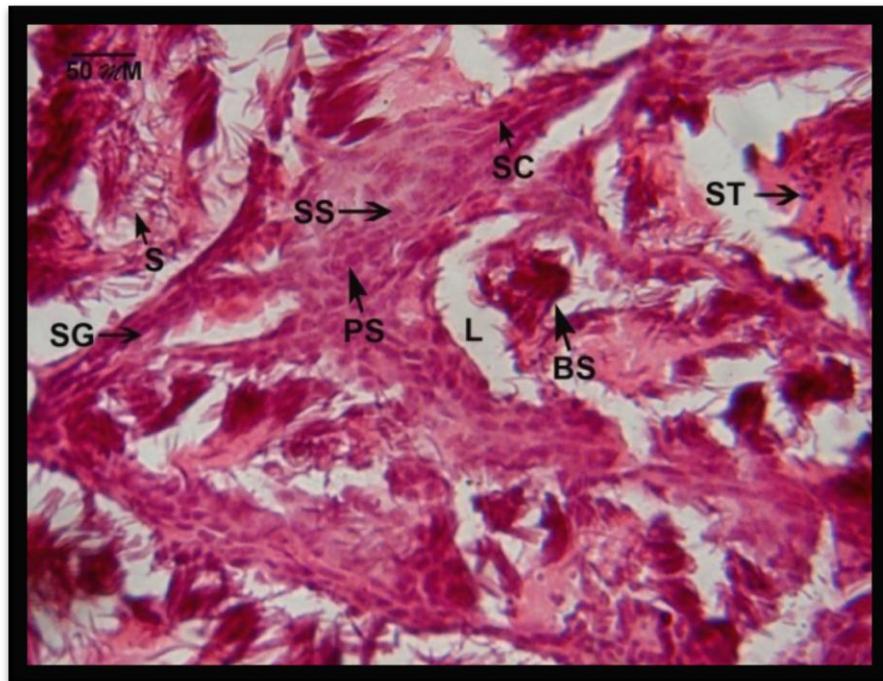


Fig. 4.53: Section of testis of *Kaloula pulchra* during the breeding season. Spermatogonia (SG), Primary spermatocytes (PS), Secondary spermatocytes (SS), Spermatids (ST), Spermatozoa (S), Bundles of spermatozoa (BS), Lumen of seminiferous tubules (L) and Sertoli cells (SC).



Fig. 4.54: Section of ovary of *Kaloula pulchra* during the breeding season. Primary oocytes (PO), Growing oocytes (GO), Animal hemisphere (AH), Vitelline membrane (V), Theca externa (TE) and Theca interna (TI).

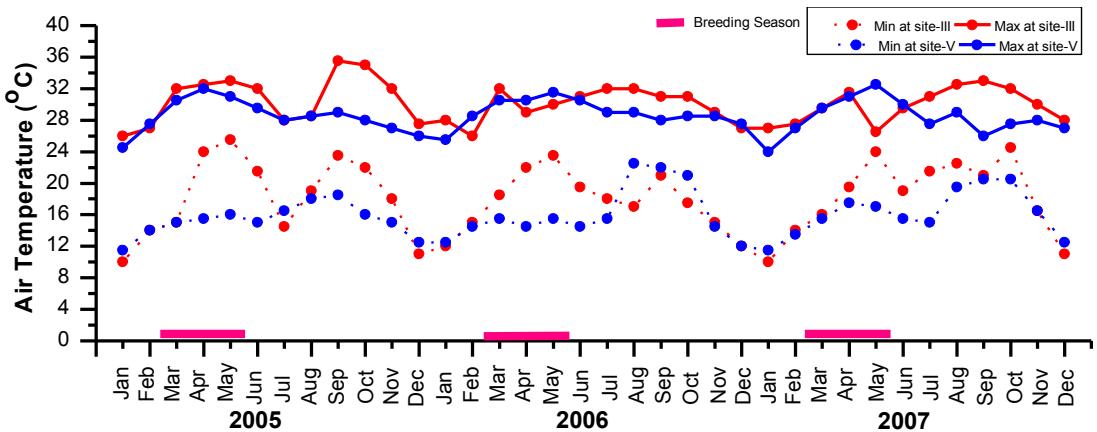


Fig. 4.55: Air temperature in the study sites III and V from 2005 to 2007

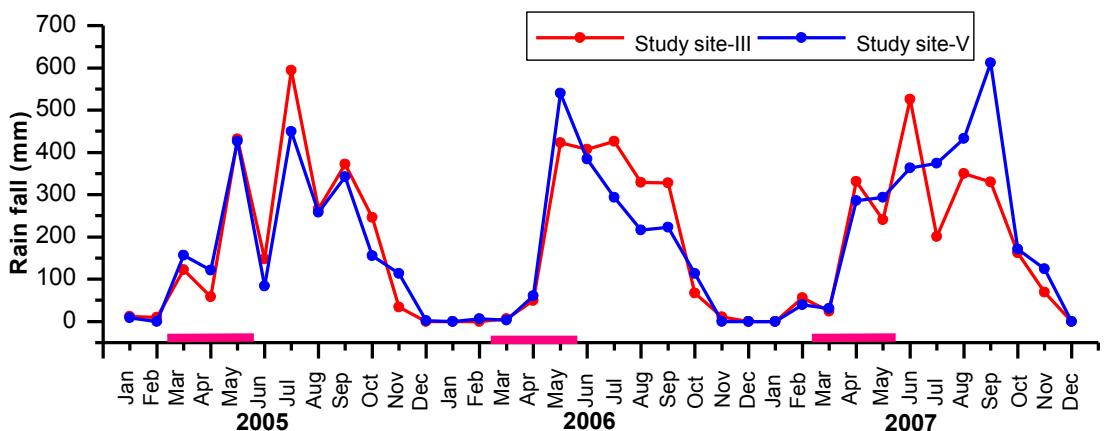


Fig. 4.56: Rainfall in the study sites III and V from 2005 to 2007

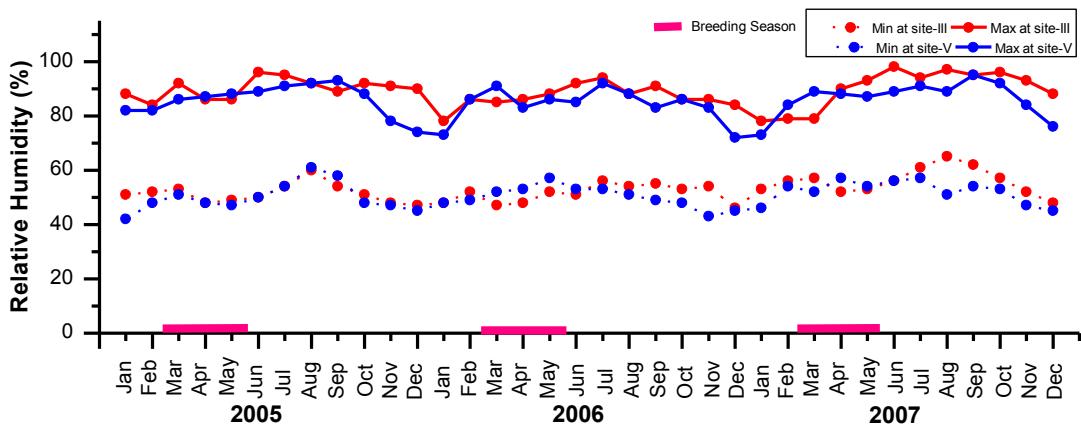


Fig. 4.57: Relative humidity in the study sites III and V from 2005 to 2007

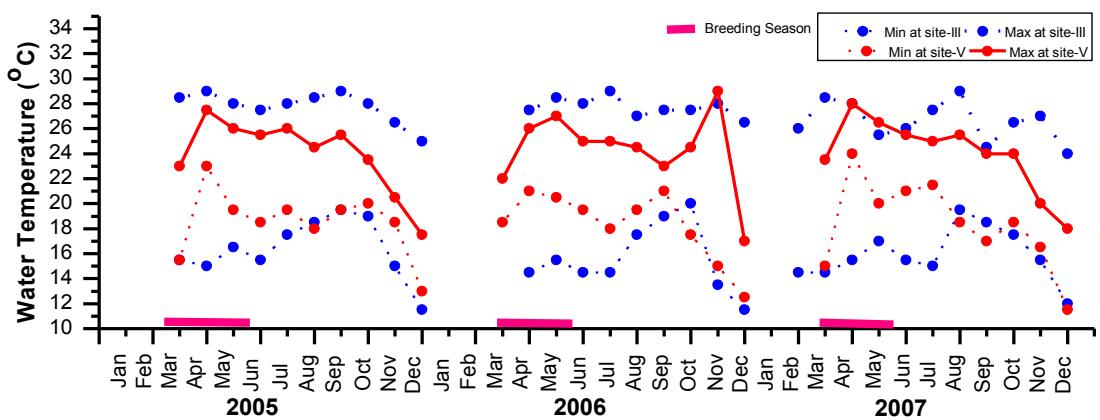


Fig. 4.58: Water temperature in the study sites III and V from 2005 to 2007

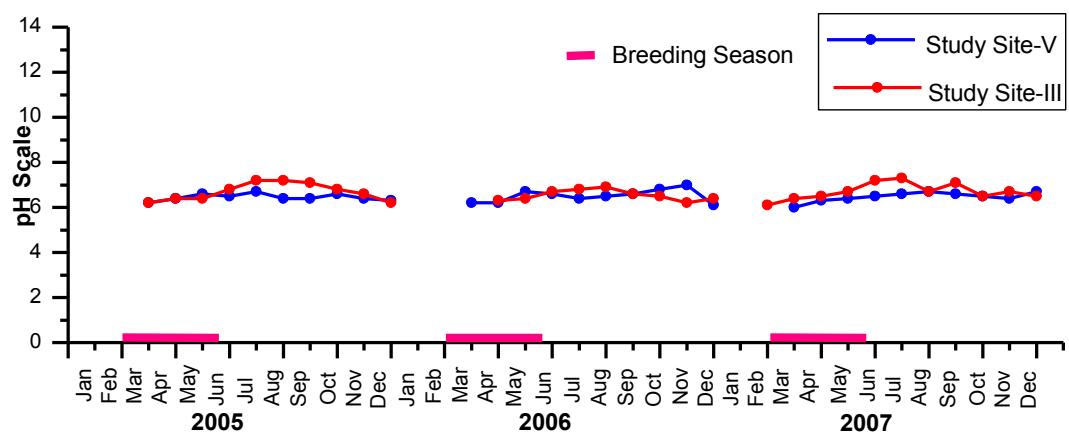


Fig. 4.59: pH of water in the study sites III and V from 2005 to 2007

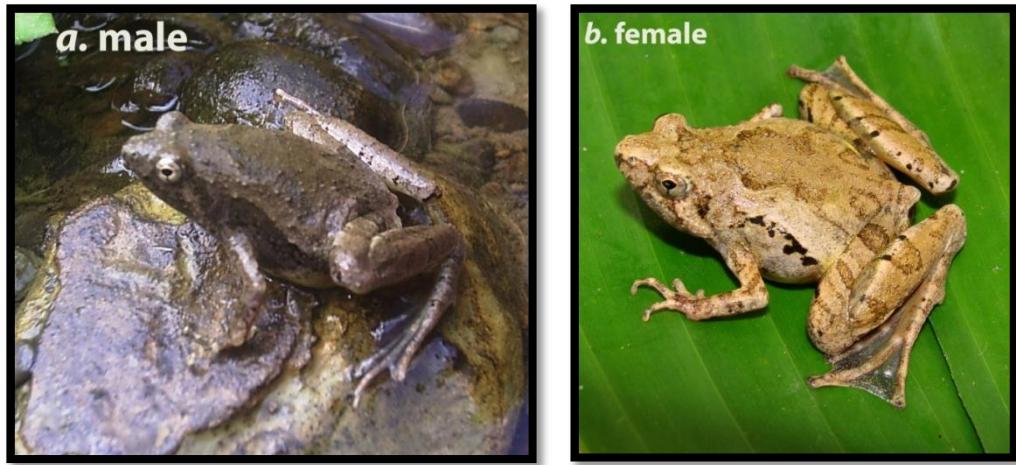


Fig. 4.60: *Microhyla berdmorei*, (a.) Male, and (b.) Female

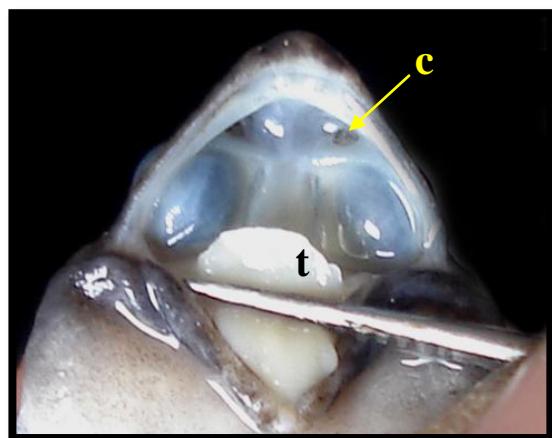


Fig. 4.61: Mouth of *Microhyla berdmorei*. t: tongue; c: chaonae.

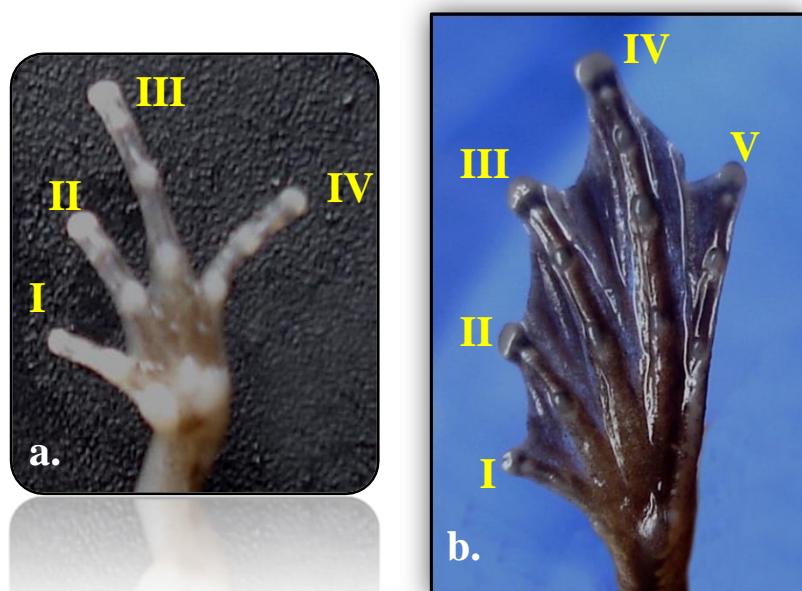


Fig. 4.62 (a.) Left hand, and (b.) left foot of *Microhyla berdmorei*
(Webbing formula: I₀₋₀II₀₋₀III₀₋₀IV₀₋₀V)



Fig. 4.63: Breeding site of *Microhyla berdmorei* at Study site-II (Tuitun stream).



Fig. 4.64: Breeding site of *Microhyla berdmorei* at Study site-IV (Tlawng river).

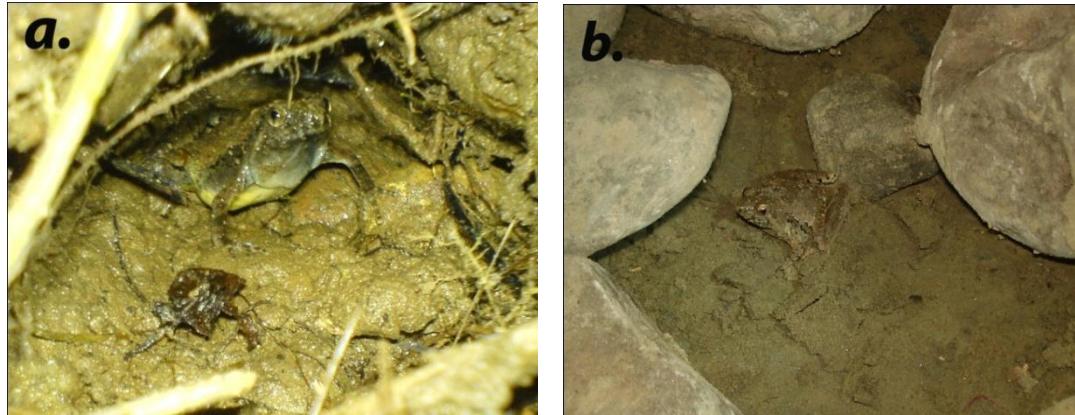


Fig. 4.65 (a&b): Males of *Microhyla berdmorei* came out to call in the evening.



Fig. 4.66(a-d): Calling of males of *Microhyla berdmorei* from different sites (a.) fissure of soil; (b.) under pebbles; (c.) twigs and (d.) muddy soil adjacent to water bodies.

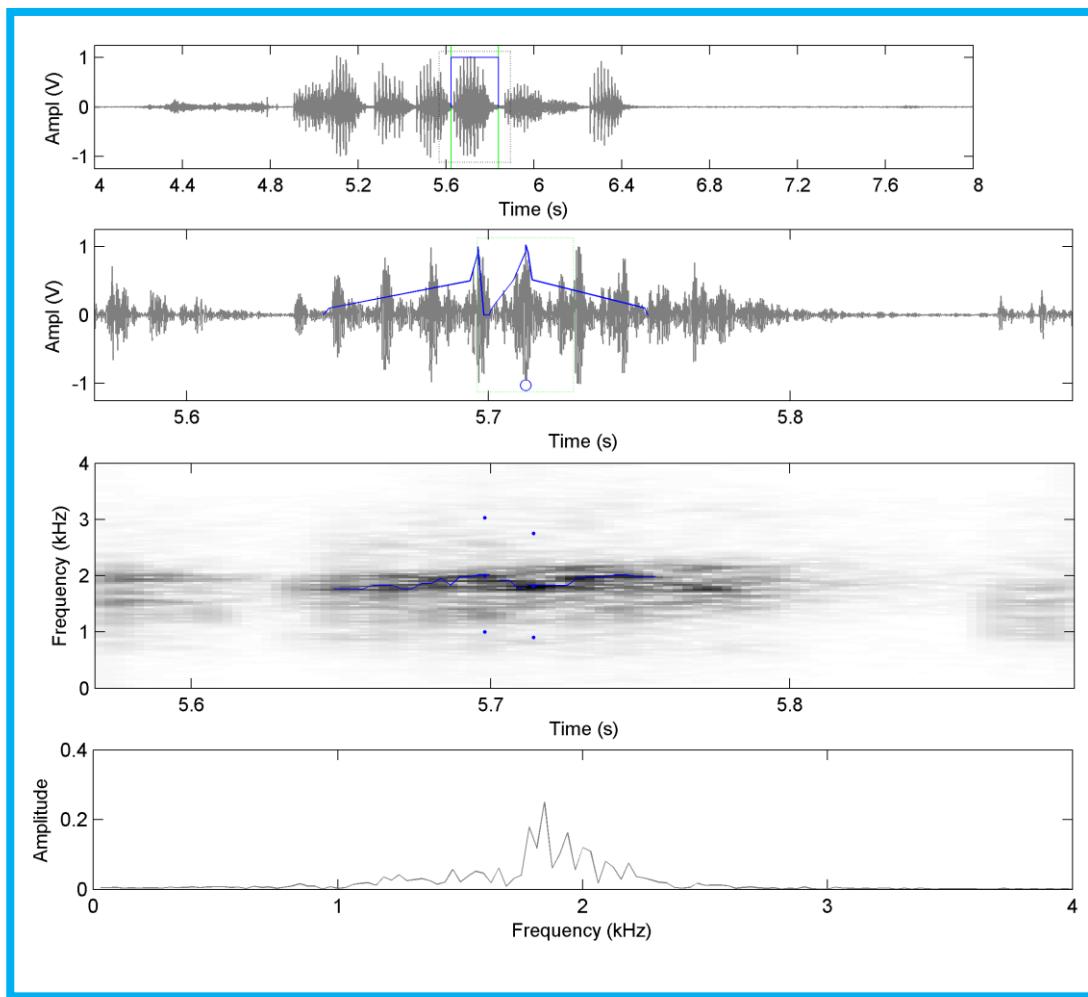


Fig. 4.67: Oscillogram, sonogram and frequency spectrum of an advertisement call of *Microhyla berdmorei*



Fig. 4.68 (a-d): Amplexing pairs of *Microhyla berdmorei*



Fig. 4.69 (a&b): Eggs clutch left by *Microhyla berdmorei*.



Fig. 4.70 (a - c): Mats of *Microhyla berdmorei*'s eggs floating on the surface of water.

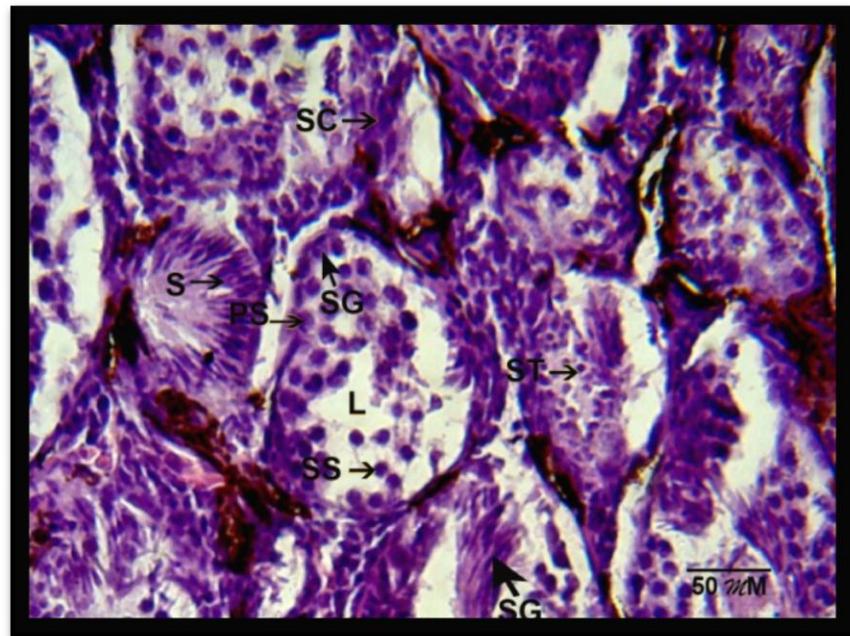


Fig. 4.71: Section of testis of *Microhyla berdmorei* during the breeding season.
Spermatogonia (SG), Primary spermatocytes (PS), Secondary spermatocytes (SS),
Spermatids (ST), Spermatozoa (S), Bundles of spermatozoa (BS), Lumen of
seminiferous tubules (L) and Sertoli cells (SC).

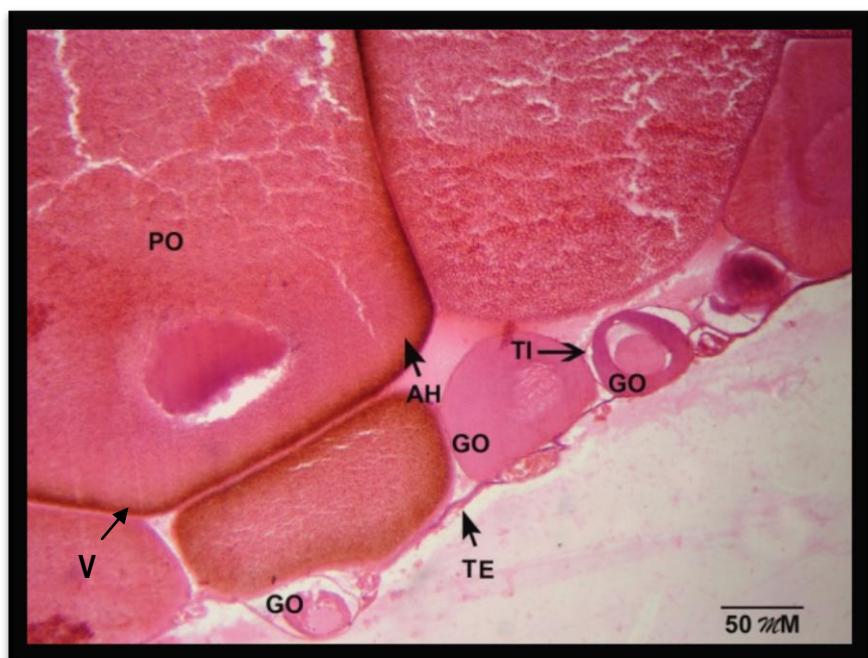


Fig. 4.72: Section of ovary of *Microhyla berdmorei* during the breeding season.
Primary oocytes (PO), Growing oocytes (GO), Animal hemisphere (AH), Vitelline
membrane (V), Theca externa (TE) and Theca interna (TI).

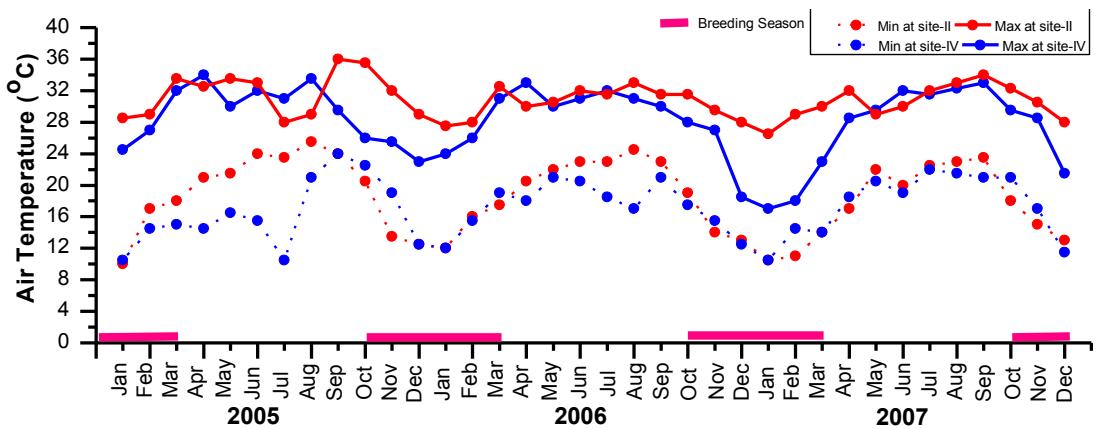


Fig. 4.73: Air temperature in the study sites II and IV from 2005 to 2007

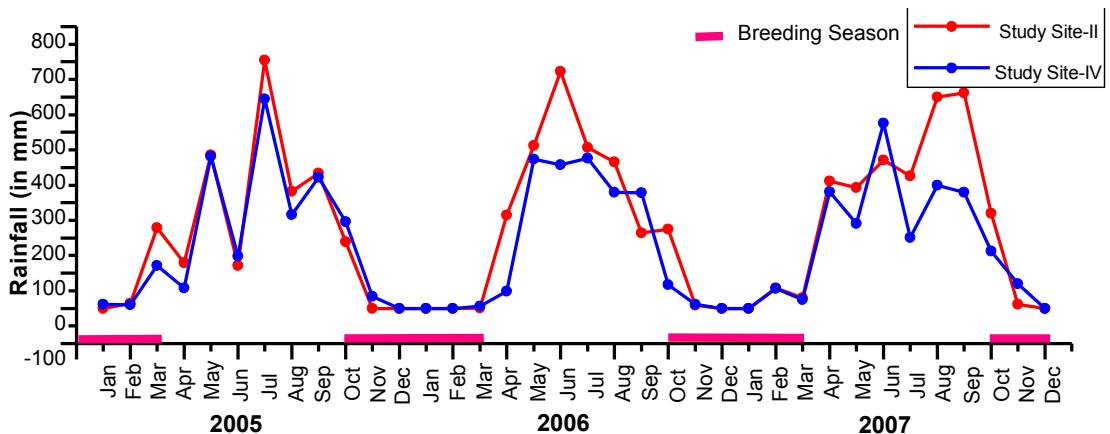


Fig. 4.74: Rainfall in the study sites II and IV from 2005 to 2007

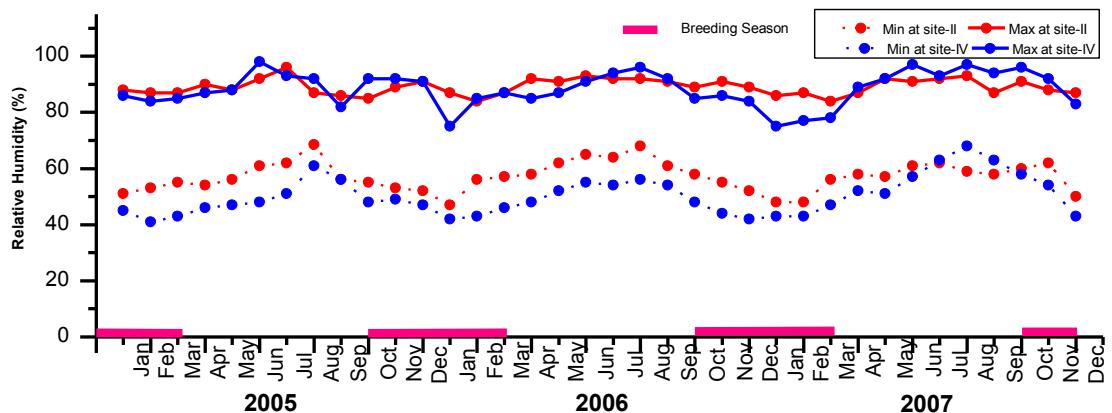


Fig. 4.75: Relative humidity in the study sites II and IV from 2005 to 2007

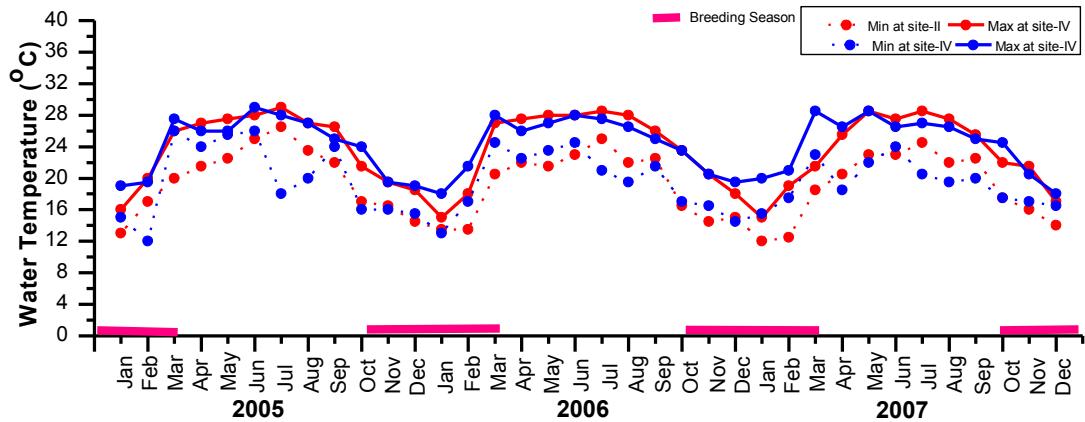


Fig. 4.76: Water temperature in the study sites II and IV from 2005 to 2007

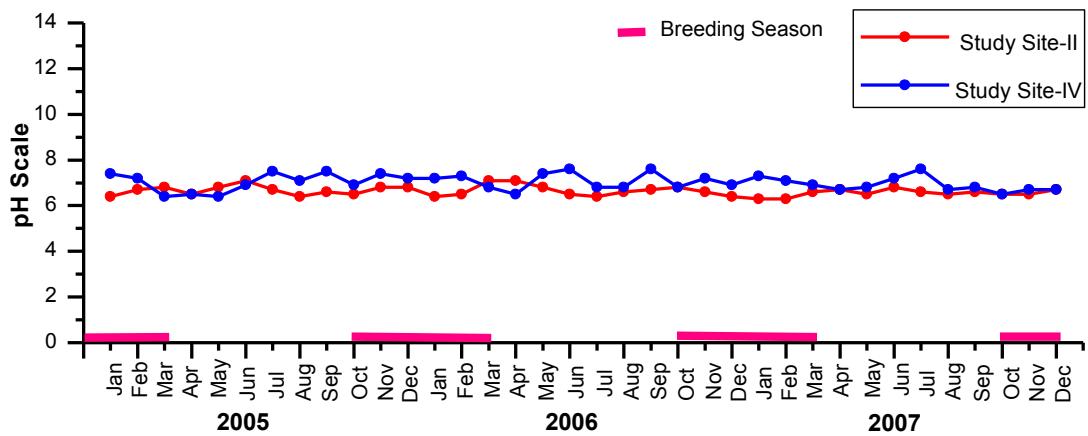


Fig. 4.77: pH of water in the study sites II and IV from 2005 to 2007

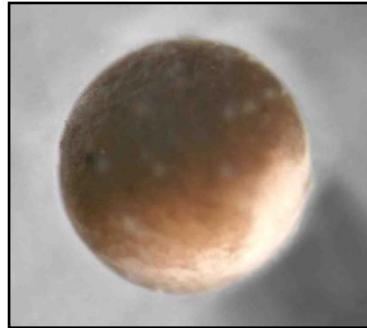


Fig.4.78.1: Stage 1 (Fertilization)

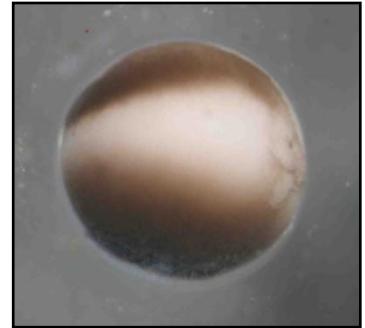


Fig.4.78.2: Stage 2 (Gray Crescent)



Fig.4.78.3: Stage 3 (2- cell)



Fig.4.78.4: Stage 4 (4 - cell)

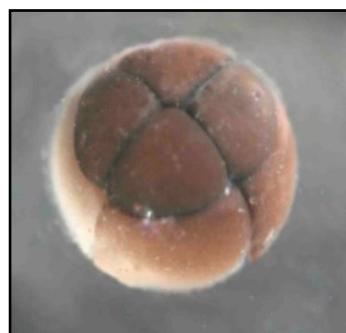


Fig.4.78.5: Stage 5 (8- cell)



Fig.4.78.6: Stage 6 (16 - cell)



Fig.4.78.7: Stage 7 (32- cell)



Fig.4.78.8 Stage 8 (Mid Cleavage)

Fig. 4.78.1-8: Developmental stages of *Euphlyctis cyanophlyctis* from stage:1 – 8.



Fig.4.78.9: Stage 9 (Late Cleavage)

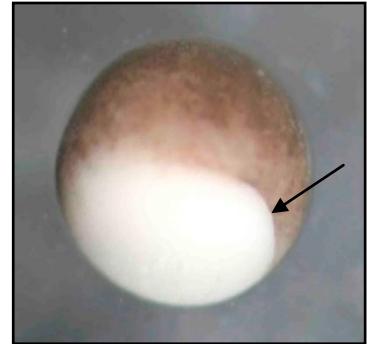


Fig.4.78.10: Stage 10 (Dorsal Lip)



Fig.4.78.11: Stage 11 (Yolk Plug)

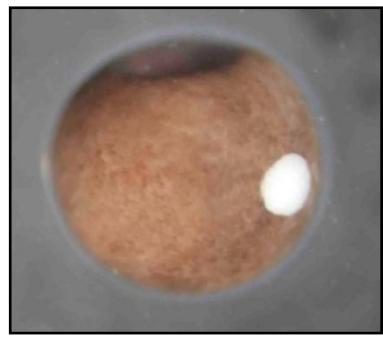


Fig.4.78.12: Stage 12 (Late Gastrula)

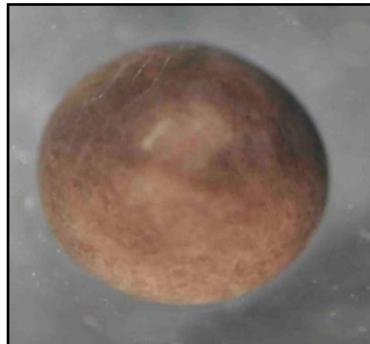


Fig.4.78.13 Stage 13 (Neural Plate)



Fig.4.78.14: Stage 14 (Neural Folds)



Fig.4.78.15: Stage 15 (Rotation)



Fig.4.78.16: Stage 16 (Neural Tube)

Fig. 4.78.9-16: Developmental stages of *Euphylyctis cyanophlyctis* from stage: 9 – 16.



Fig.4.78.17: Stage 17 (Tail Bud)



Fig.4.78.18: Stage 18 (Muscular Response)



Fig.4.78.19: Stage 19 (Heart Beat)

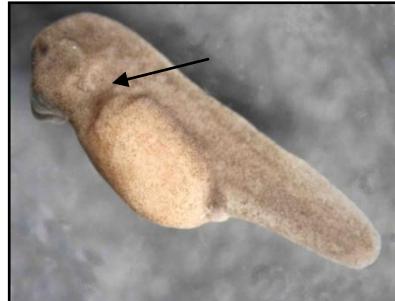


Fig.4.78.20: Stage 20 (Gill Circulation)

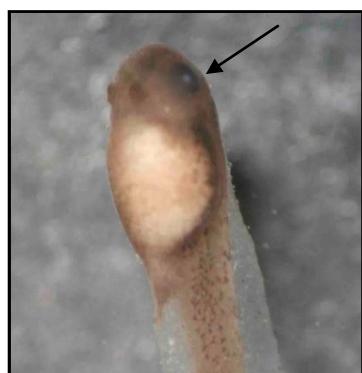


Fig.4.78.21: Stage 21 (Cornea Transparent) Fig.4.78.22: Stage 22 (Fin Circulation)

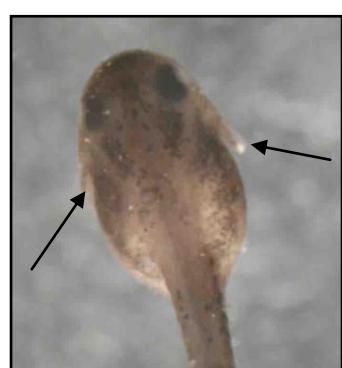
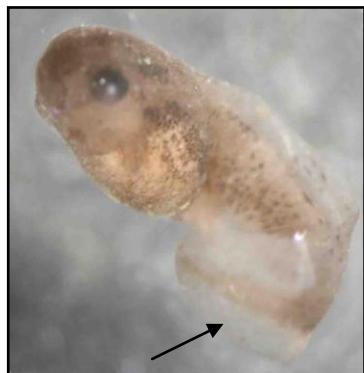


Fig.4.78.23: Stage 23 (Operculum present) Fig.4.78.24: Stage 24 (Left Gill)

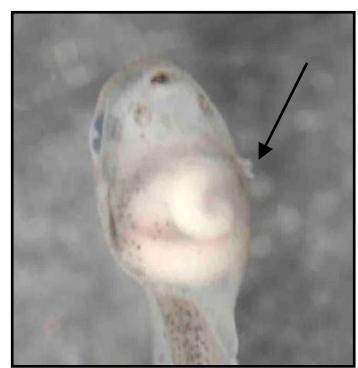


Fig.4.78.17-24:Developmental stages of *Euphylyctis cyanophlyctis* from stage:17 - 24

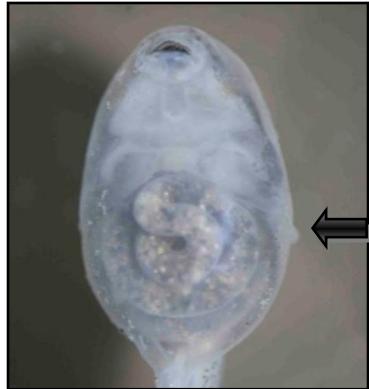


Fig.4.78.25: Stage 25 (Left spiracle)

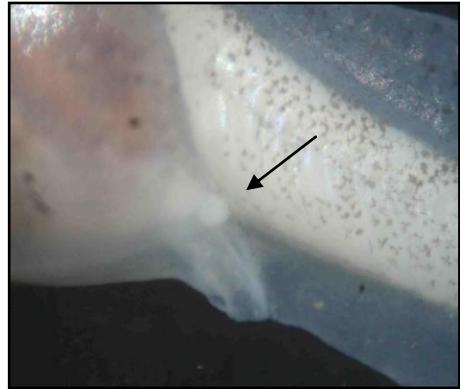


Fig.4.78.26: Stage 26 ($L < 1/2D$)

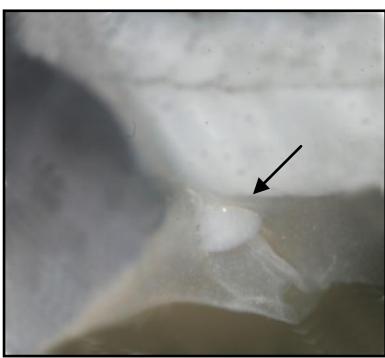


Fig.4.78.27: Stage 27 ($L \geq 1/2D$)

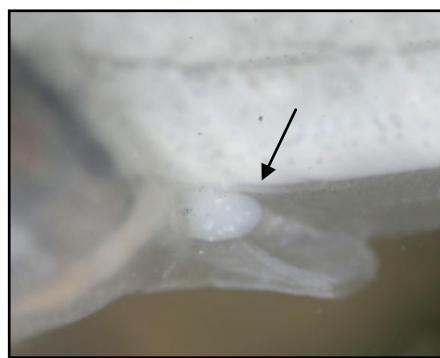


Fig.4.78.28: Stage 28($L \geq D$)

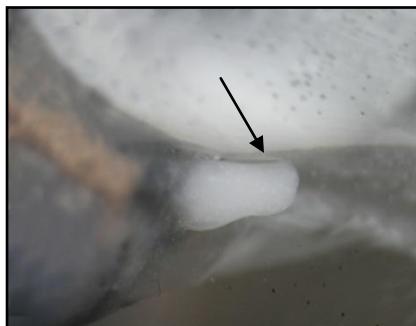


Fig.4.78.29: Stage 29 ($L \geq 1_{1/2}D$)

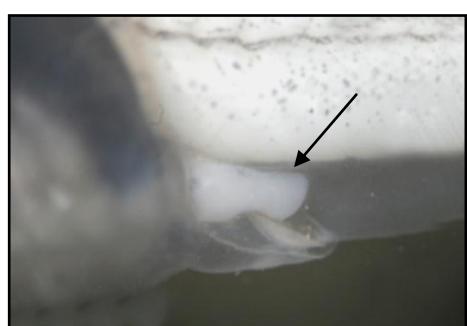


Fig.4.78.30a: Stage 30($L = 2D$)



Fig.4.78.30b: Stage 30 (Whole body in dorsal view)

Fig.4.78.25-30: Developmental stages of *Euphlyctis cyanophlyctis* from stage: 25-30

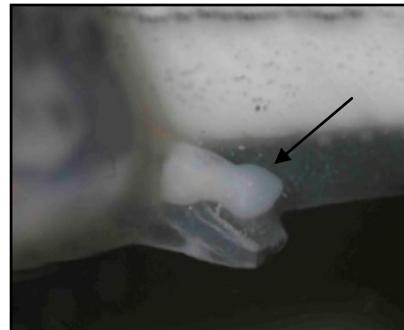


Fig.4.78.31: Stage 31 (Foot Paddle)

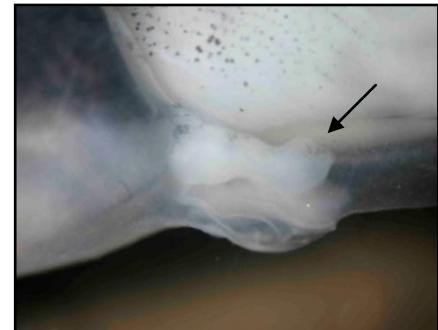


Fig.4.78.32: Stage 32 (Indentation 4 -5)



Fig.4.78.33: Stage 33 (Indentation 3-4)



Fig.4.78.34a: Stage 34 (Indentation 2-3)



Fig.4.78.34b: Stage 34 (Whole body in lateral view)



Fig.4.78.35: Stage 35 (Indentation 1-2)

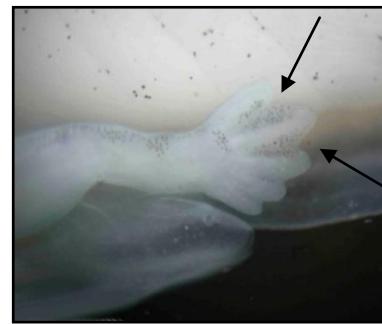


Fig.4.78.36: Stage 36 (Toes 3-5) separated

Fig. 4.78.31-36: Developmental stages of *Euphlyctis cyanophlyctis* from stage:31– 36



Fig.4.78.37: Stage 37
Toes Separated



Fig.4.78.38: Stage 38
Metatarsal Tubercles

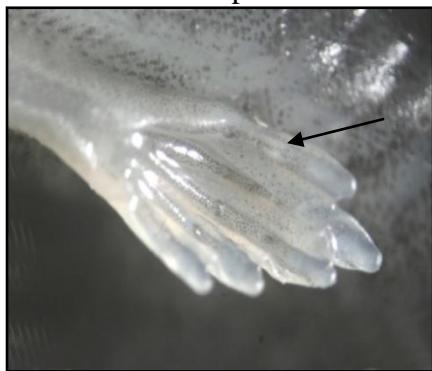


Fig.4.78.39: Stage 39
Sub-articular patches

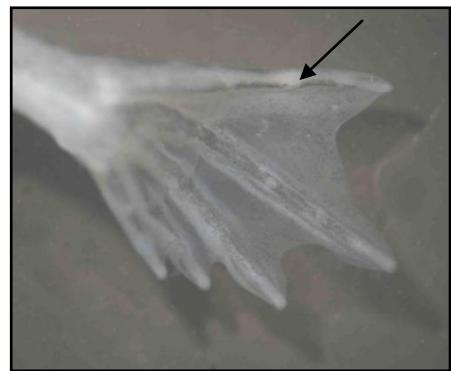


Fig.4.78.40a: Stage 40
Foot Tubercles



Fig.4.78.40b: Stage 40 (Whole body in dorsal view)

Fig.4.78.57-40: Developmental stages of *Euphlyctis cyanophlyctis* from stage:37 - 40

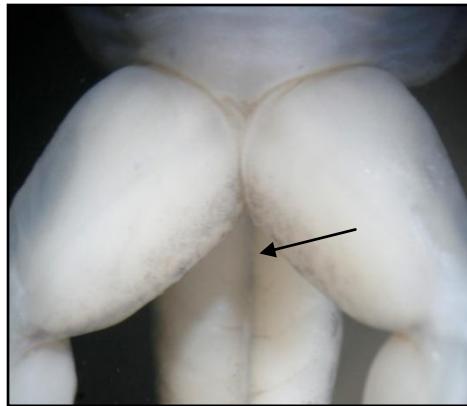


Fig.4.78.41: Stage 41
Vent Tube Gone

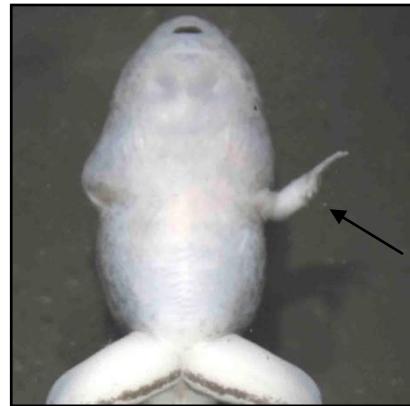


Fig.4.78.42: Stage 42
Fore-limbs Emerge

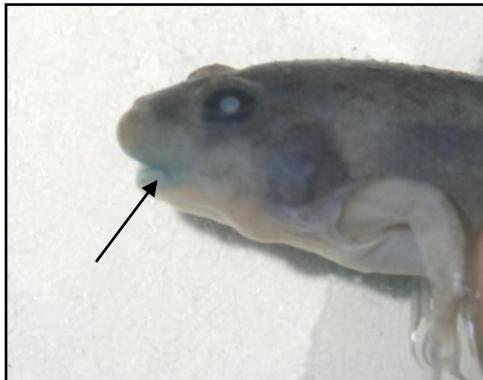


Fig.4.78.43: Stage 43
Mouth Between Nostril and Eye

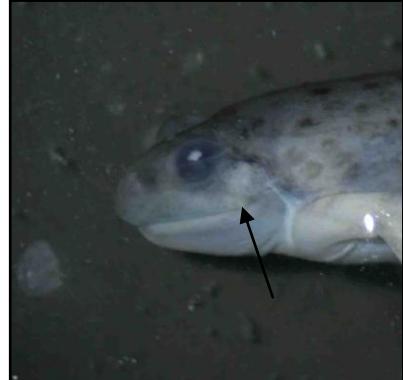


Fig.4.78.44: Stage 44
Mouth Beneath Eye

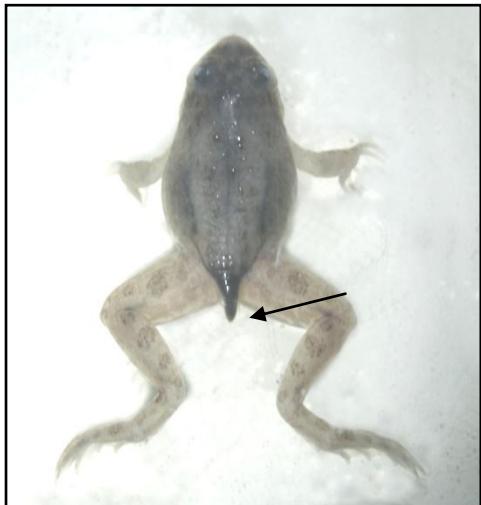


Fig.4.78.45: Stage 45
Tail Stub



Fig.4.78.46: Stage 46
Metamorphosis Complete

Fig. 4.78.41-46: Developmental stages of *Euphlyctis cyanophlyctis* from stage: 41-46



Fig.4.79.1: Stage 1 (Fertilization)



Fig.4.79.2: Stage 2 (Gray Crescent)



Fig.4.79.3: Stage 3 (2- cell)

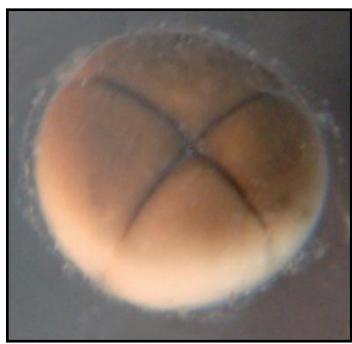


Fig.4.79.4: Stage 4 (4 – cell)

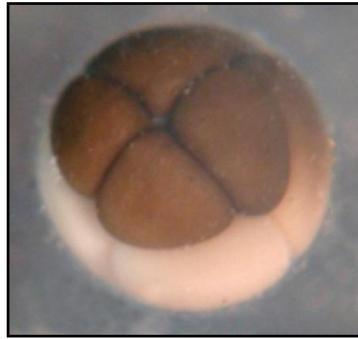


Fig.4.79.5: Stage 5 (8- cell)

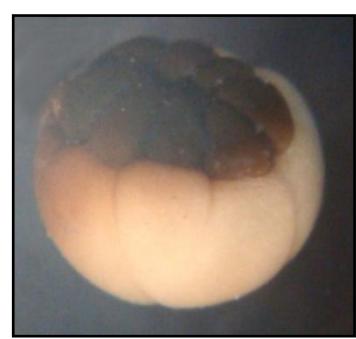


Fig.4.79.6: Stage 6 (16 - cell)

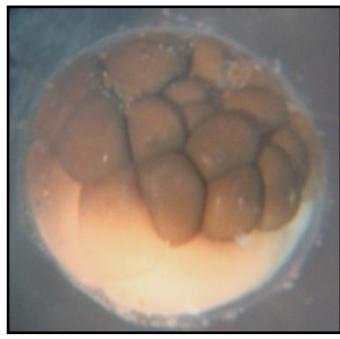


Fig.4.79.7: Stage 7 (32- cell)



Fig.4.79.8: Stage 8 (Mid Cleavage)

Fig. 4.79.1-8: Developmental stages of *Hylarana nicobariensis* from stage-1 to stage-8

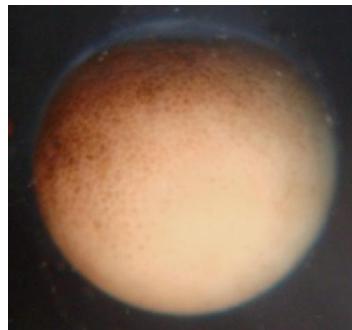


Fig.4.79.9: Stage 9 Late Cleavage

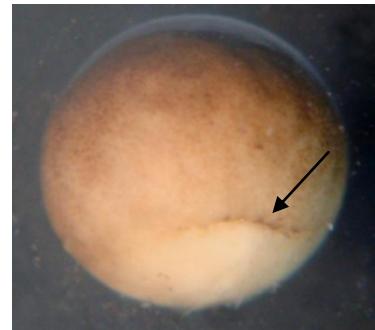


Fig.4.79.10: Stage 10 Dorsal Lip

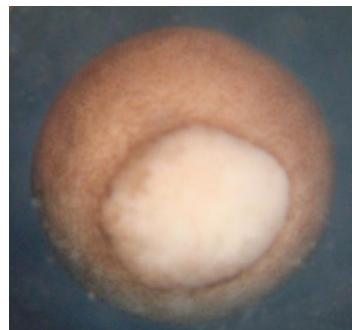


Fig.4.79.11: Stage Yolk Plug 11

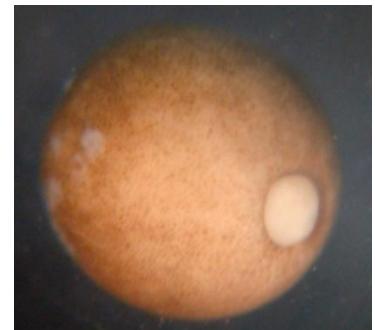


Fig.4.79.12: Stage 12 Late Gastrula



Fig.4.79.13: Stage 13 Neural Plate



Fig.4.79.14: Stage 14 Neural Folds



Fig.4.79.15: Stage 15 Rotation



Fig.4.79.16: Stage 16 Neural Tube

Fig. 4.79.9-16: Developmental stages of *Hylarana nicobariensis* from stage-9 to stage-16



Fig.4.79.17: Stage 17 Tail Bud



Fig.4.79.18: Stage 18 Muscular Response



Fig.4.79.19: Stage 19 Heart Beat

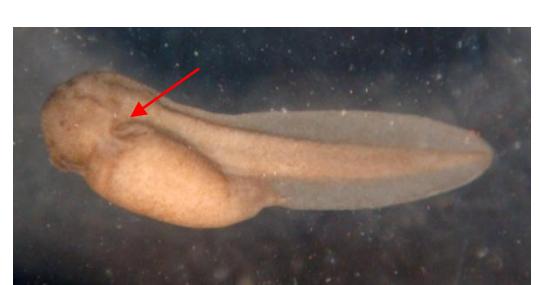


Fig.4.79.20: Stage 20 Gill Circulation



Fig.4.79.21: Stage 21 Cornea Transparent



Fig.4.79.22: Stage 22 Fin Circulation



Fig.4.79.23: Stage 23
Operculum present

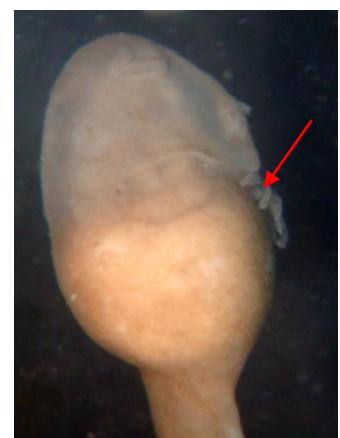


Fig.4.79.24: Stage 24
Left Gill



Fig.4.79.25: Stage 25
Spiracle forms on left

Fig. 4.79.17-25: Developmental stages of *Hylarana nicobariensis* from stage-17 to stage-25



Fig.4.79.26a: Stage 26 Whole body in dorsal view



Fig.4.79.26b: Stage 26 Hind Limb Bud ($L < 1/2D$)



Fig.4.79.27: Stage 27 ($L \geq 1/2D$)



Fig.4.79.28: Stage 28 ($L \geq D$)



Fig.4.79.29: Stage 29($L \geq 1\frac{1}{2}D$)



Fig.4.79.30: Stage 30 ($L = 2D$)



Fig.4.79.31: Stage 31 Foot paddle

Fig. 4.79.26a-31: Developmental stages of *Hylarana nicobariensis* from stage-26a to stage-31



Fig.4.79.32: Stage 32 Indentation 4-5



Fig.4.79.33a: Stage 33 Indentation 3-4



Fig.4.79.33b: Stage 33 Whole body in dorsal view



Fig.4.79.34: Stage 34 Indentation 2-3



Fig.4.79.35: Stage 35 Indentation 1-2



Fig.4.79.36: Stage 36: 3-5 Toes Separated



Fig.4.79.37a: Stage 37 Toes Separated

Fig. 4.79.32-37a: Developmental stages of *Hylarana nicobariensis* from stage-32 to stage-37



Fig.4.79.37b: Stage 37 (Whole body in lateral view)



Fig.4.79.38:Stage38 Metatarsal tubercles



Fig.4.79.39a:Stage39 Sub-articular patches



Fig.4.79.39b: Stage 39 Whole body in dorsal view

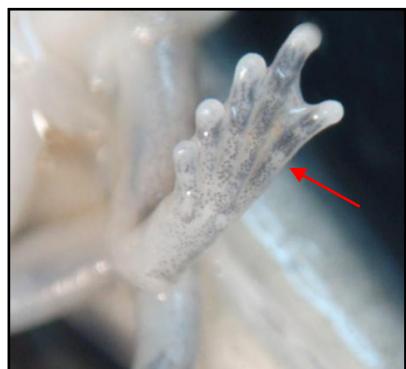


Fig.4.79.40:Stage Foot 40 Tuberclcs

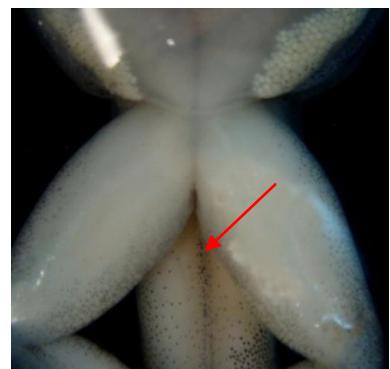


Fig.4.79.41:Stage 41 Vent-Tube Gone

Fig. 4.79.37b-41: Developmental stages of *Hylarana nicobariensis* from stage-37 to stage-41



Fig.4.79.42: Stage 42
Fore Limb Emerge

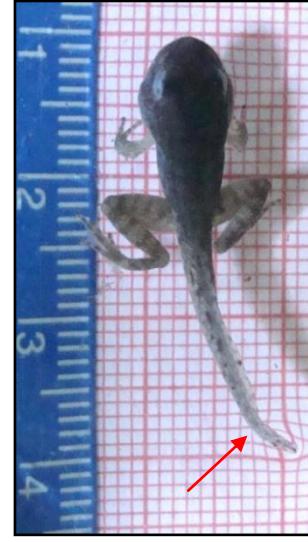


Fig.4.79.43: Stage 43
Tail Atrophies

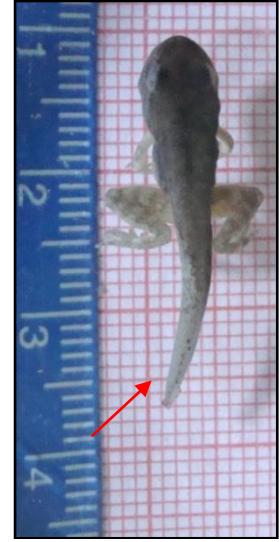


Fig.4.79.44: Stage 44
Tail Greatly Reduced

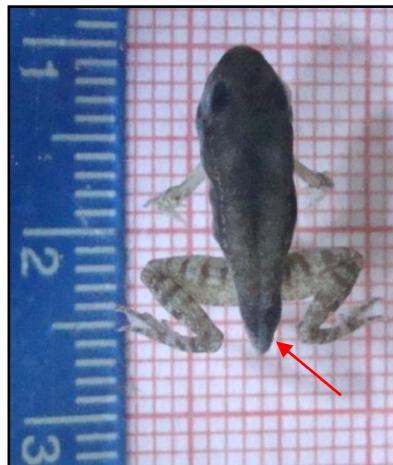


Fig.4.79.45: Stage 45 Tail Stub



Fig.4.79.46a: Stage 46 Froglet



Fig.4.79.46b: Emerging froglet in the field

Fig. 4.79.42-46b: Developmental stages of *Hylarana nicobariensis* from stage-42 to stage-46

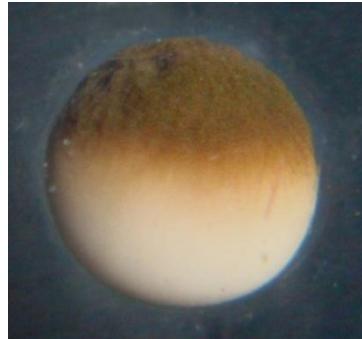


Fig.4.80.1: Stage 1 Fertilization



Fig.4.80.2: Stage 2 Gray Crescent



Fig.4.80.3: Stage 3: 2-cell

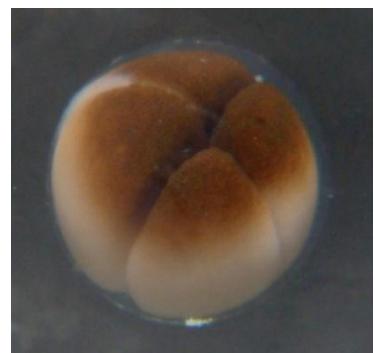


Fig.4.80.4: Stage 4: 4 - cell



Fig.4.80.5: Stage 5: 8- cell



Fig.4.80.6: Stage 6: 16 - cell



Fig.4.80.7: Stage 7: 32- cell



Fig.4.80.8: Stage 8: Mid Cleavage

Fig. 4.80.1-8: Developmental stages of *Kaloula pulchra* from stage-1 to stage-8

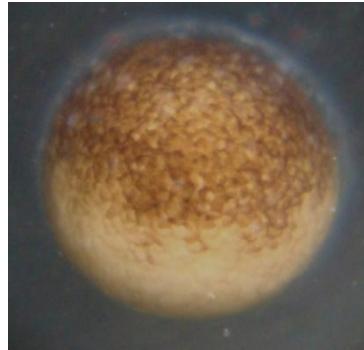


Fig.4.80.9: Stage 9: Late Cleavage



Fig.4.80.10: Stage 10 Dorsal Lip

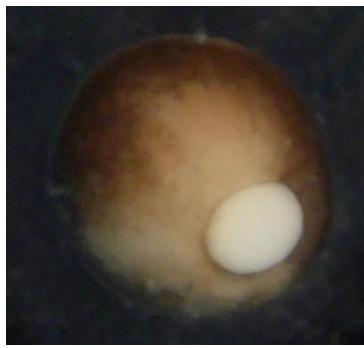


Fig.4.80.11: Stage 11: Yolk Plug

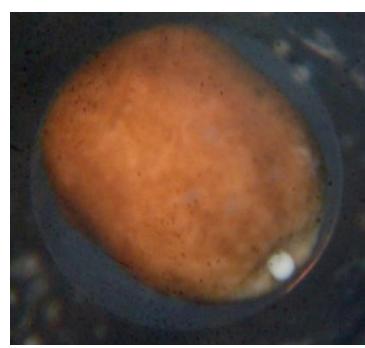


Fig.4.80.12: Stage 12: Late Gastrula



Fig.4.80.13: Stage 13: Neural Plate



Fig.4.80.14: Stage 14: Neural Folds



Fig.4.80.15: Stage 15: Rotation



Fig.4.80.16: Stage 16: Neural Tube

Fig. 4.80.9-16: Developmental stages of *Kaloula pulchra* from stage-9 to stage-16



Fig.4.80.17: Stage 17: Tail Bud



Fig.4.80.18: Stage 18: Muscular Response



Fig.4.80.19: Stage 19: Heart Beat



Fig.4.80.20: Stage 20: Gill Circulation



Fig.4.80.21: Stage 21: Cornea Transparent



Fig.4.80.22: Stage 22: Fin Circulation



Fig.4.80.23: Stage 23
Operculum covers gill base



Fig.4.80.24: Stage 24
Left Gill

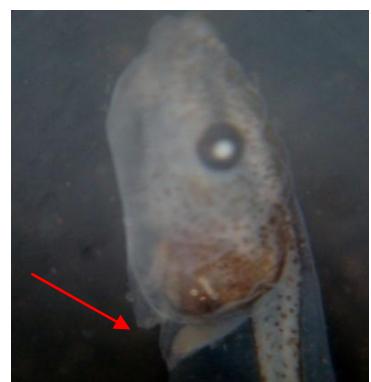


Fig.4.80.25: Stage 25
Spiracle Forms on Mid-Ventral

Fig. 4.80.17-25: Developmental stages of *Kaloula pulchra* from stage-17 to stage-25



Fig.4.80.26a: Stage 26: Dorsal view

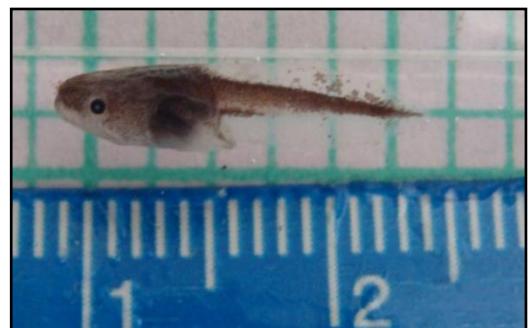


Fig.4.80.26b: Stage 26: Lateral view



Fig.4.80.26c: Stage 26: $L < \frac{1}{2}D$



Fig.4.80.27: Stage 27: $L \geq \frac{1}{2}D$



Fig.4.80.28: Stage 28 $L \geq D$



Fig.4.80.29: Stage 29 $L \geq 1\frac{1}{2}D$

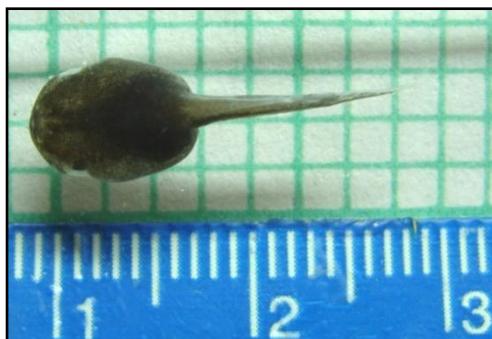


Fig.4.80.30a: Stage 30: Dorsal view

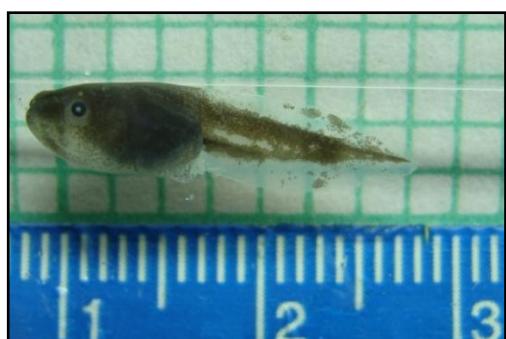


Fig.4.80.30b: Stage 30: Lateral view

Fig.4.80.26a-30b: Developmental stages of *Kaloula pulchra* from stage:26-30

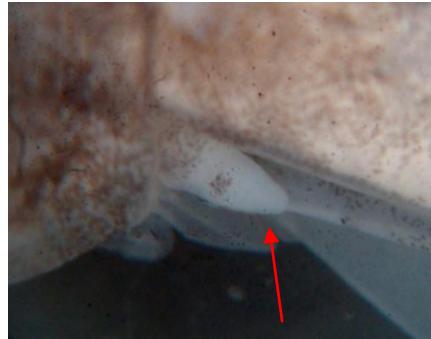


Fig.4.80.30c: Stage 30: $L \geq 2D$



Fig.4.80.31: Stage 31: Foot Paddle



Fig.4.80.32: Stage 32: Indentation 4-5



Fig.4.80.33: Stage 33: Indentation 3-4



Fig.4.80.34a: Stage 34: Dorsal view

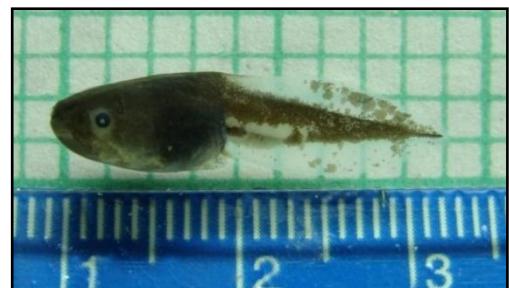


Fig.4.80.34b: Stage 34: Lateral view



Fig.4.80.34c: Stage 34: Indentation 2-3



Fig.4.80.35: Stage 35: Indentation 1-2

Fig. 4.80.30c-35: Developmental stages of *Kaloula pulchra* from stage-30 to stage-35



Fig.4.80.36: Stage 36: Toes 3-5 Separated



Fig.4.80.37: Stage 37: All Toes Separated



Fig.4.80.38:Stage 38: Metatarsal tubercles



Fig.4.80.39: Stage 39:Sub-articular patches



Fig.4.80.40: Stage 40: Foot Tubercl es

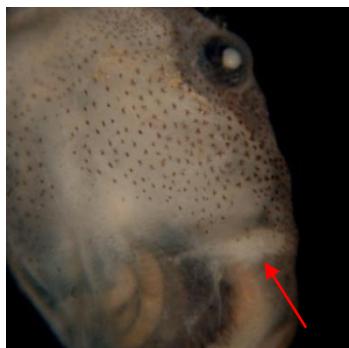


Fig.4.80.41: Stage 41: Fore Limbs Visible



Fig.4.80.42(a&b): Stage 42: (a.) Right Fore Limb Emerge first (b.)Fore Limbs Emerge



Fig. 4.80.36-42a&b: Developmental stages of *Kaloula pulchra* from stage: 36 – 42.



Fig.4.80.43: Stage 43: Tail Atrophies



Fig.4.80.44: Stage 44: Tail Greatly Reduced



Fig.4.80.45: Stage 45: Tail Stub



Fig.4.80.46a: Stage 46:Metamorphosis Complete



Fig.4.80.46b: Emerging froglets and metamorphs in the field.

Fig. 4.80.43-46b: Developmental stages of *Kaloula pulchra* from stage: 43 – 46.



Fig.4.81.1: Stage 1: Fertilization



Fig.4.81.2: Stage 2: Gray Crescent



Fig.4.81.3: Stage 3: 2- cell



Fig.4.81.4: Stage 4: 4 - cell



Fig.4.81.5: Stage 5: 8- cell



Fig.4.81.6: Stage 6: 16 - cell



Fig.4.81.7: Stage 7: 32- cell



Fig.4.81.8: Stage 8: Mid Cleavage

Fig. 4.81.1-8: Developmental stages of *Microhyla berdmorei* from stage-1 to stage-8



Fig.4.81.9: Stage 9: Late Cleavage



Fig.4.81.10: Stage 10 Dorsal Lip



Fig.4.81.11: Stage 11: Yolk Plug



Fig.4.81.12: Stage 12: Late Gastrula



Fig.4.81.13: Stage 13: Neural Plate



Fig.4.81.14: Stage 14: Neural Folds



Fig.4.81.15: Stage 15: Rotation



Fig.4.81.16: Stage 16: Neural Tube

Fig. 4.81.8-16: Developmental stages of *Microhyla berdmorei* from stage: 8 – 16.



Fig.4.81.17: Stage 17: Tail Bud



Fig.4.81.18: Stage 18: Muscular Response



Fig.4.81.19: Stage 19: Heart Beat



Fig.4.81.20: Stage 20: Gill Circulation

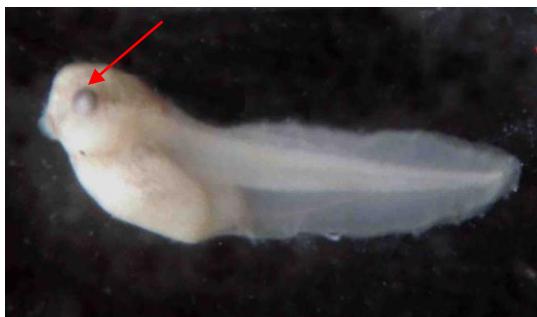


Fig.4.81.21:Stage 21:Cornea Transparent



Fig.4.81.22:Stage 22:Tail Fin Circulation

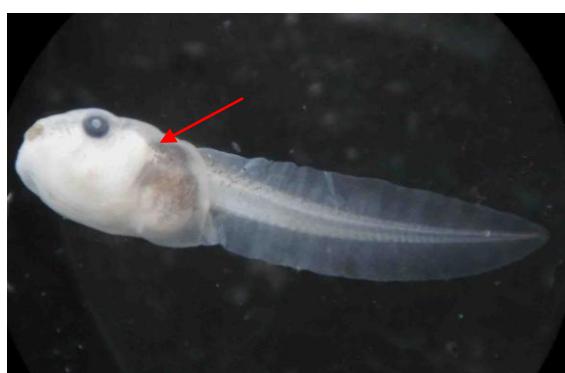


Fig.4.81.23: Stage 23: Operculum present



Fig.4.81.24: Stage 24: Left Gill

Fig. 4.81.17-24: Developmental stages of *Microhyla berdmorei* from stage:17 – 24.

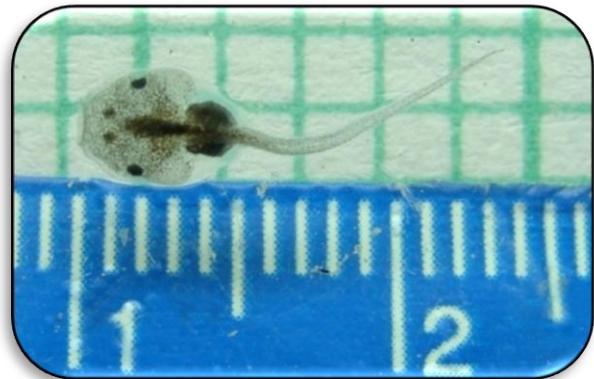


Fig.4.81.25a: Stage 25: Whole body in dorsal view



Fig.4.81.25b: Stage 25: Ventro-medial Spiracle



Fig.4.81.26: Stage 26: $L < 1/2D$



Fig.4.81.27: Stage 27: $L \geq 1/2D$



Fig.4.81.28: Stage 28: $L \geq D$

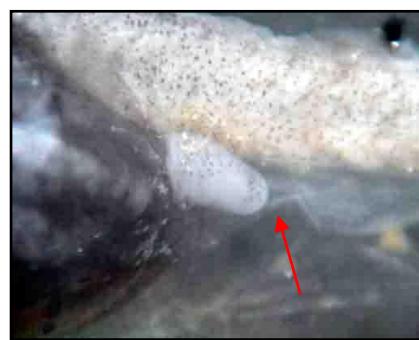


Fig.4.81.29: Stage 29: $L \geq 1\frac{1}{2}D$



Fig.4.81.30a: Stage 30: $L = 2D$

Fig. 4.81.25a-30a: Developmental stages of *Microhyla berdmorei* from stage:25 – 30.

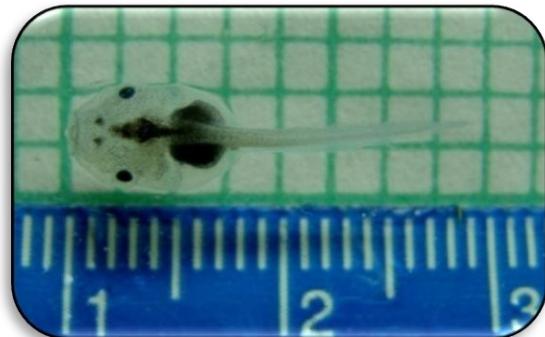


Fig.4.81.30b: Stage 30: Whole body in dorsal view

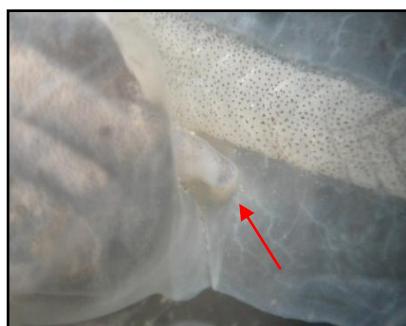


Fig.4.81.31: Stage 31: Foot Paddle



Fig.4.81.32: Stage 32: Indentation 4 -5



Fig.4.81.33: Stage 33: Indentation 3-4



Fig.4.81.34a: Stage 34: Indentation 2-3



Fig.4.81.34b: Stage 34: Whole body in dorsal view

Fig. 4.81.30b-34b:Developmental stages of *Microhyla berdmorei* from stage:30– 34.



Fig.4.81.35: Stage 35: Indentation 1-2

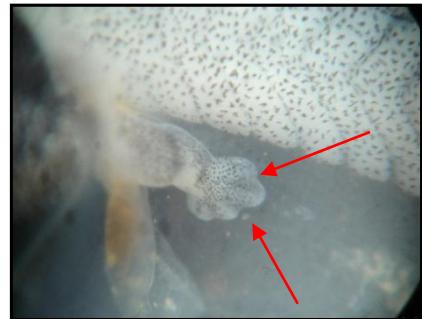


Fig.4.81.36: Stage 36: Toes 3-5 Separated

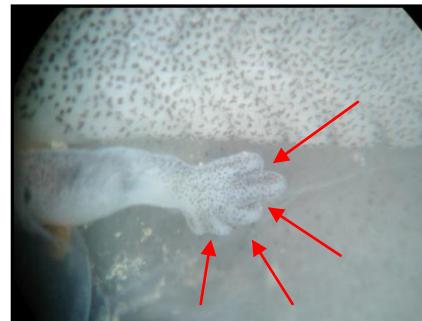


Fig.4.81.37:Stage37:All Toes Separated



Fig.4.81.38a:Stage 38:Metatarsal Tubercles



Fig.4.81.38b: Stage 38: Whole body in lateral view

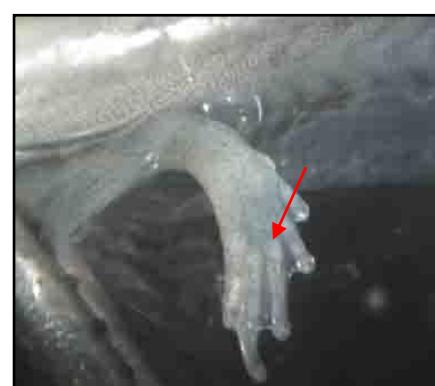


Fig.4.81.39: Stage 39: Sub-articular patches



Fig.4.81.40: Stage 40:Foot Tuberclles

Fig. 4.81.35-40: Developmental stages of *Microhyla berdmorei* from stage:35– 40.



Fig.4.81.41:Stage 41:Fore-limb visible

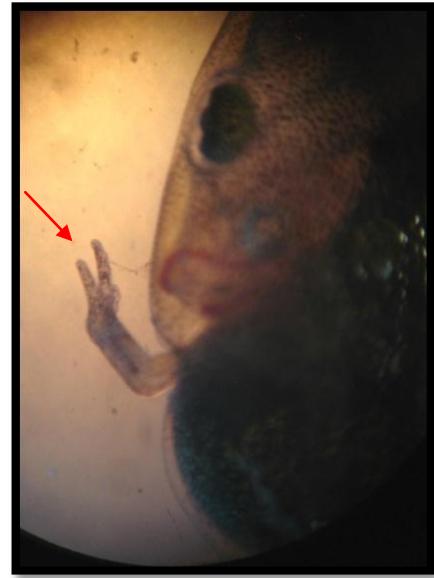


Fig.4.81.42:Stage 42:Fore-limb Emerge

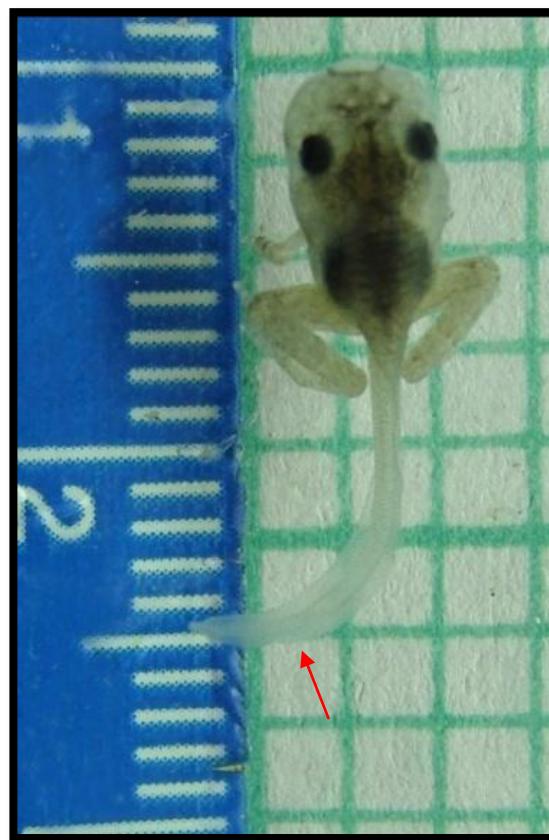


Fig.4.81.43: Stage 43: Tail Atrophies

Fig. 4.81.41-43: Developmental stages of *Microhyla berdmorei* from stage: 41–43.

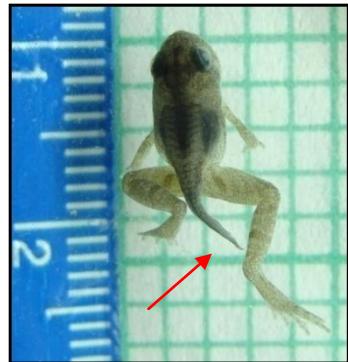


Fig.4.81.44: Stage 44
Tail Greatly Reduced

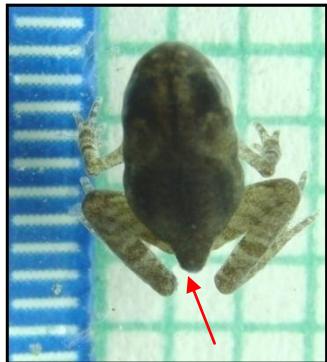


Fig.4.81.45: Stage 45
Tail stub



Fig.4.81.46a: Stage 46
Metamorphosis Complete



Fig.4.81.46b: Emerging froglets start to come out from water

Fig. 4.81.44 - 46b: Developmental stages of *Microhyla berdmorei* from stage:44-46.



Fig. 4.82: Benthic microhabitat of tadpoles of *Euphlyctis cyanophlyctis*

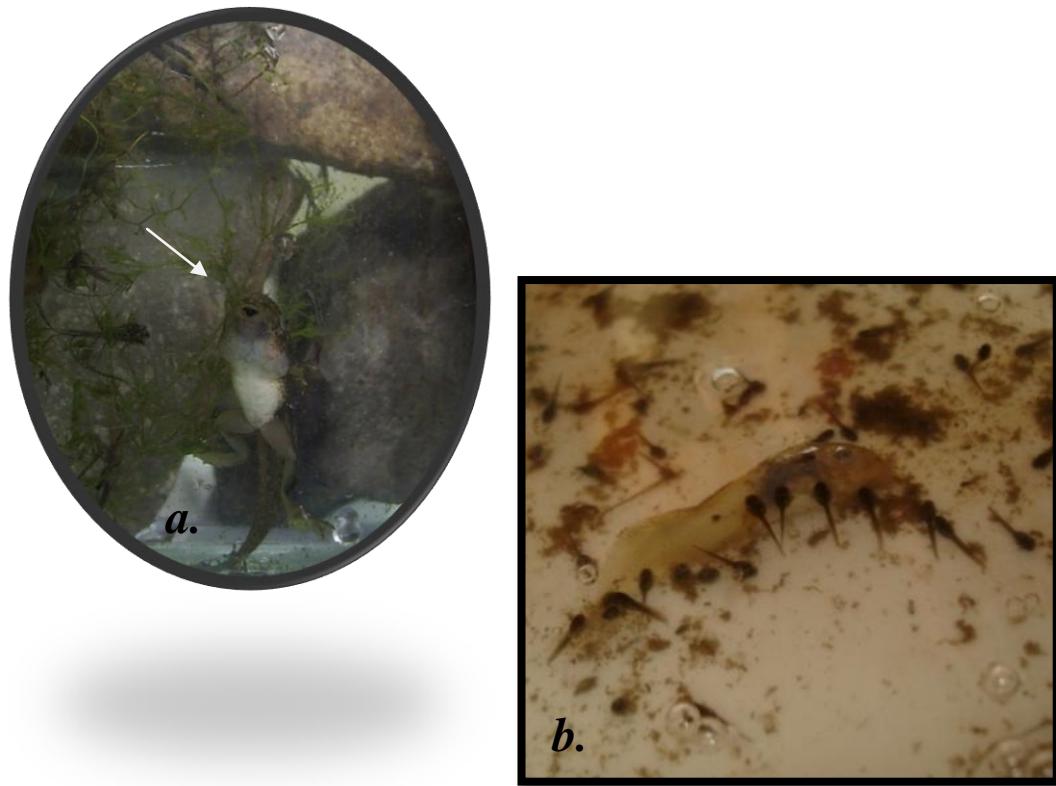


Fig. 4.83(a&b): Feeding behavior of tadpoles of *Euphlyctis cyanophlyctis* (a.) Facultative suspension-feeders as well as (b.) larvivorous.

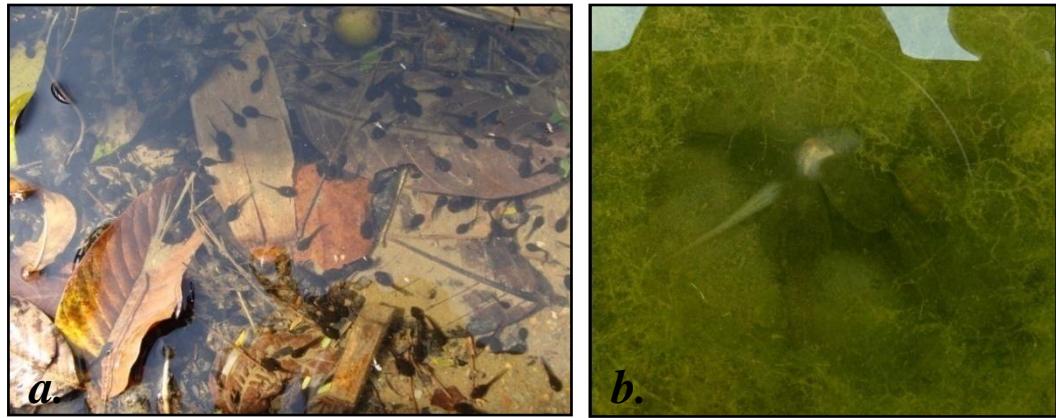


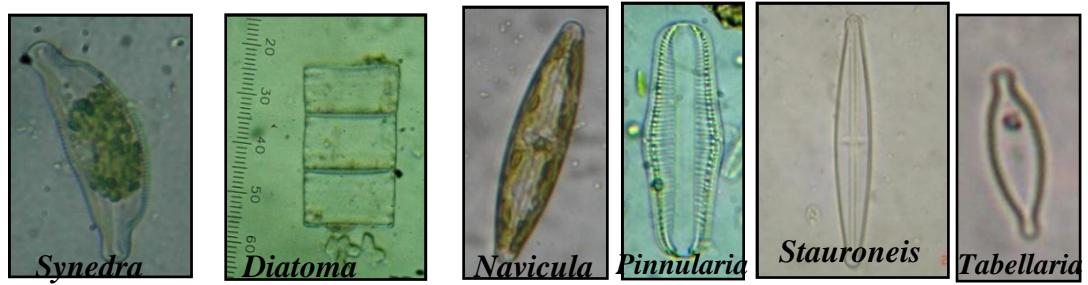
Fig. 4.84(a&b): Feeding behavior of tadpoles of *Hylarana nicobariensis* (a.) Facultative suspension-feeders as well as (b.) larvivorous.



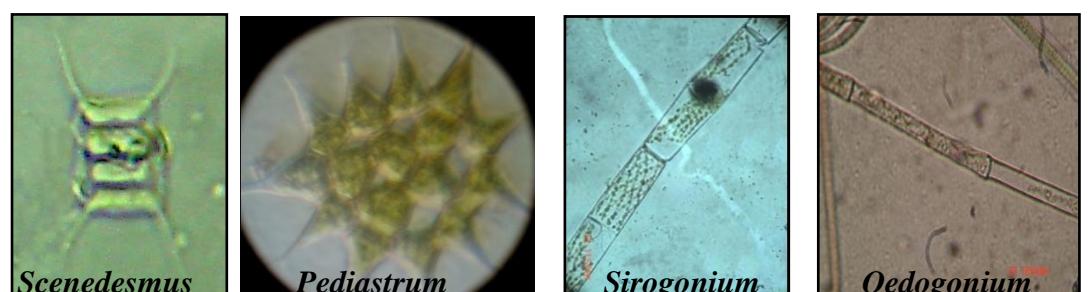
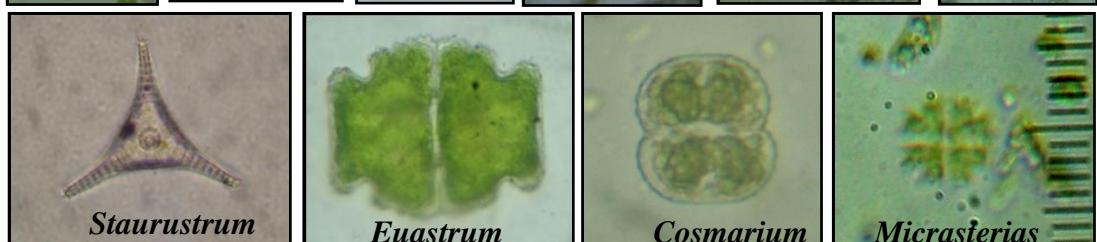
Fig. 4.85(a&b): Feeding behavior of tadpoles of *Kaloula pulchra* (a.) rock-pools microhabitats and (b.) they are obligate suspension-feeders distributed throughout the water column.



Fig. 4.86(a&b): Feeding behavior of tadpoles of *Microhyla berdmorei* (a.) shady lentic microhabitats and (b.) they are distributed throughout the water column and obligate suspension-feeders



a. Bacillariophyceae

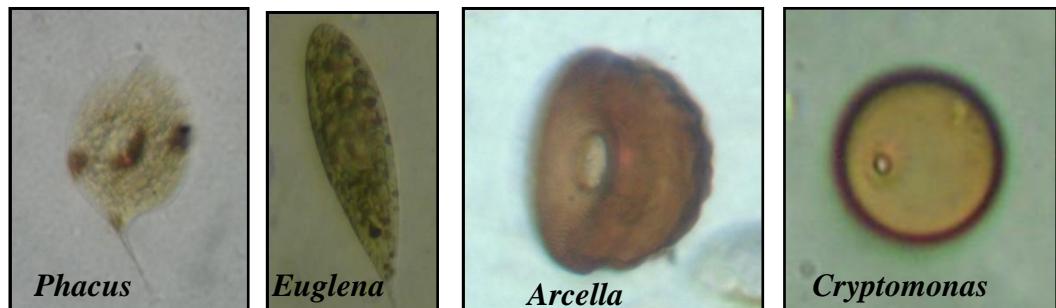


b. Chlorophyceae



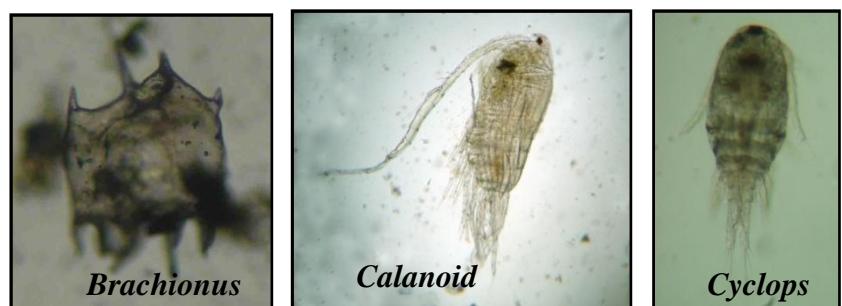
c. Cyanophyceae

Fig. 4.87(a&c.): Phytoplankton found in the intestines of tadpoles



d. Euglenophyceae

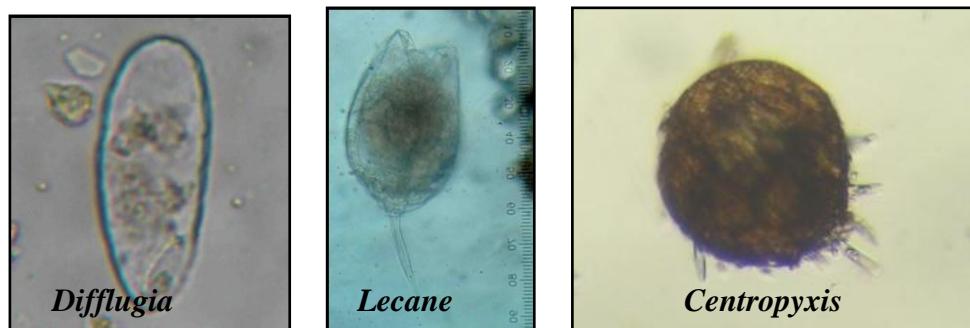
e. Cryptophyceae



Brachionus

Calanoid

Cyclops



Diffugia

Lecane

Centropyxis



Nauplius larva

f. Zooplankton

Fig. 4.87(d&e.): Non-phytoplankton found in the intestines of tadpoles

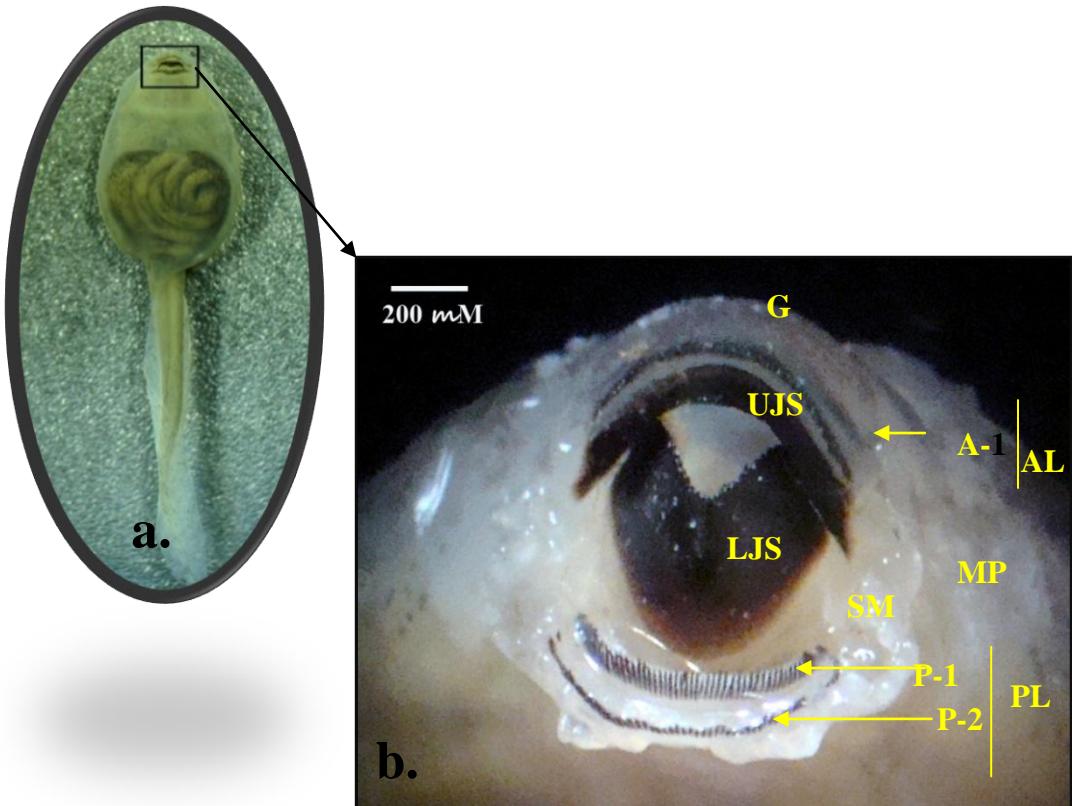


Fig. 4.88(a&b): Light micrographs of stage-25 of *Euphlyctis cyanophlyctis* (a.) whole ventral body and (b.) oral apparatus. LTRF (Labial Teeth Row Formula) = $\frac{1}{2}$

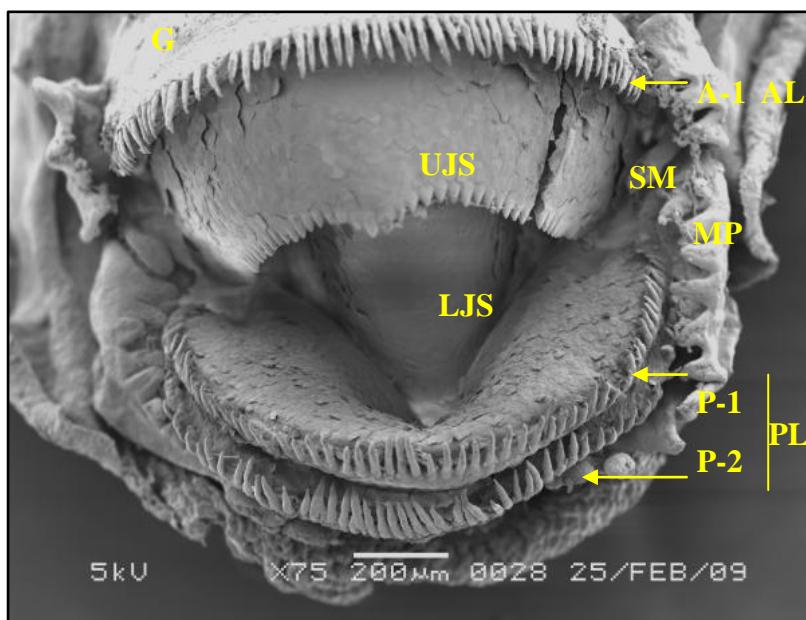


Fig. 4.89: Scanning electron micrograph of oral apparatus of stage 25 of *Euphlyctis cyanophlyctis*. A-1: Anterior tooth row 1; P-1: Posterior tooth row 1; P-2: Posterior tooth row; AL: Anterior labium; PL: Posterior labium; SM: Submarginal papillae; G: Dorsal gap.

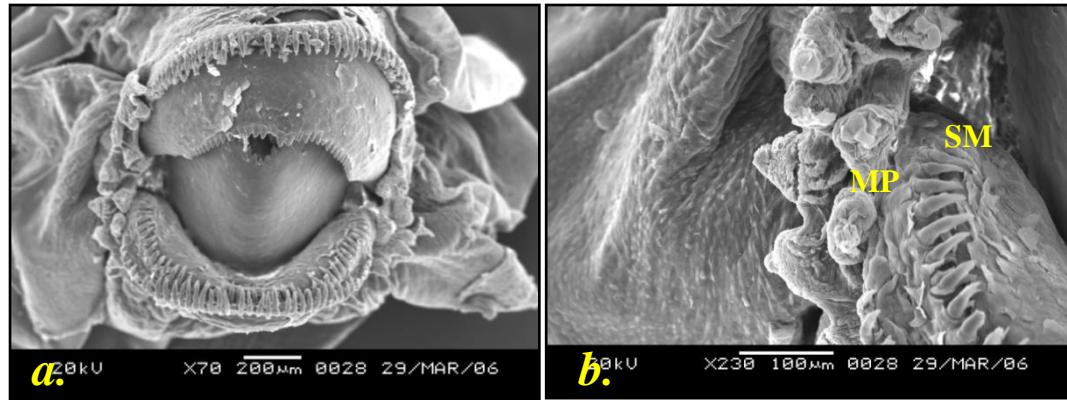


Fig. 4.90(a&b): (a.) Scanning electron micrograph of oral apparatus at stage 28 of *Euphlyctis cyanophlyctis*, and (b.) close up view of marginal papillae (MP) & sub marginal papillae (SM).

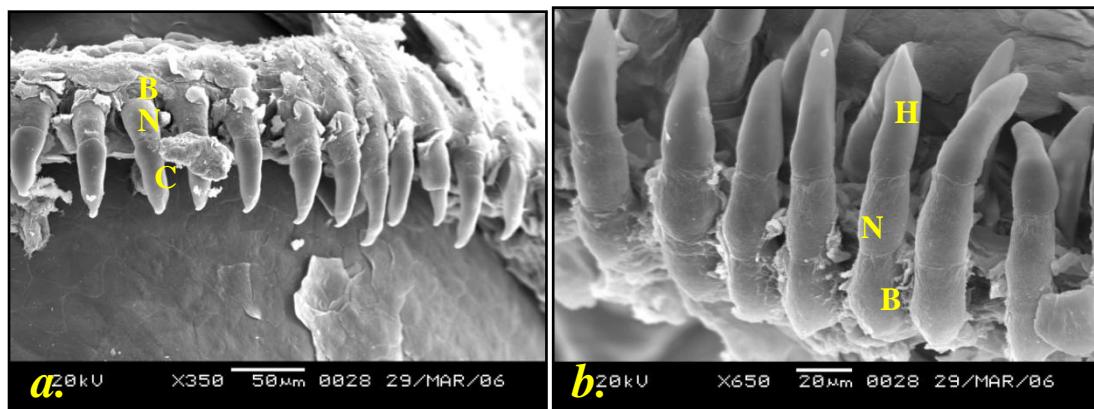


Fig. 4.91(a&b): Scanning electron micrograph of (a.) upper labium, and (b.) lower labium showing teeth rows at stage 28 of *Euphlyctis cyanophlyctis*. B: Base; N: Neck; H: Head.

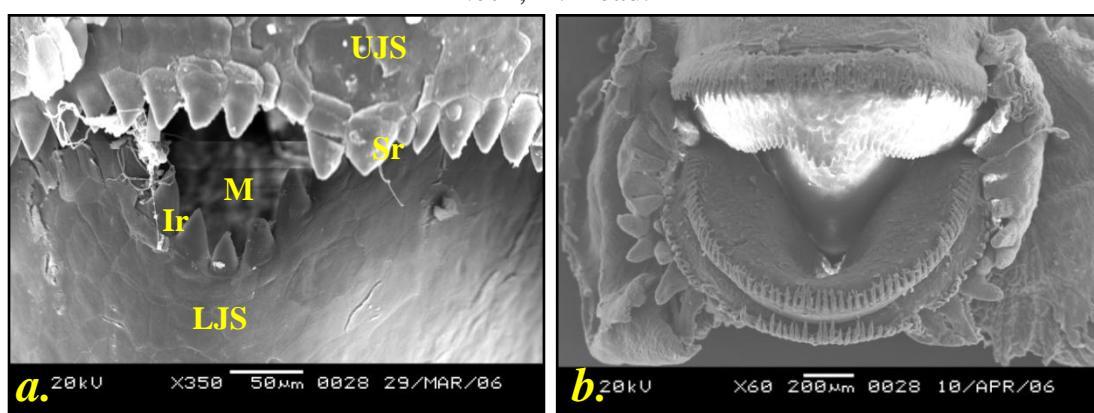


Fig. 4.92(a&b): Scanning electron micrographs of oral structure of *Euphlyctis cyanophlyctis* at (a.) stage 28 showing UJS, LJS, mouth (M), suprarostrodont (Sr) and infrarostrodont (Ir), and (b.) stage 37 showing the same LTRF

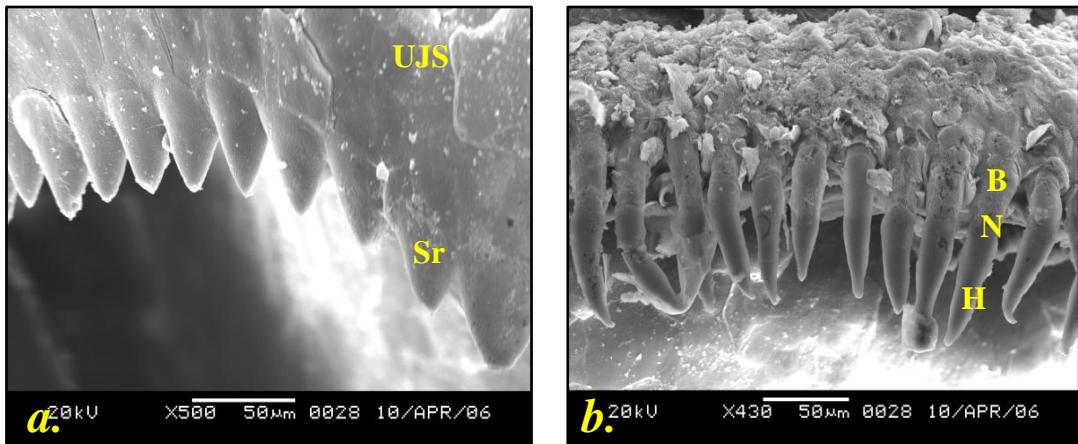


Fig. 4.93(a&b): Scanning electron micrographs of oral structures of *Euphlyctis cyanophlyctis* at stage 37, (a.) upper jaw sheath, and (b.) upper labium with a single keratodont row.

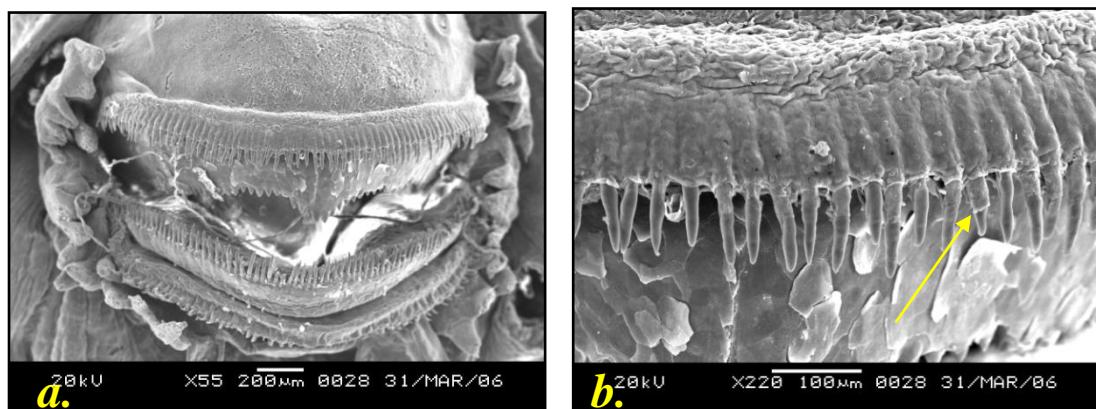


Fig. 4.94(a&b): Scanning electron micrographs of oral structures of *Euphlyctis cyanophlyctis* at stage 40, (a.) whole apparatus, and (b.) degenerating anterior tooth row.

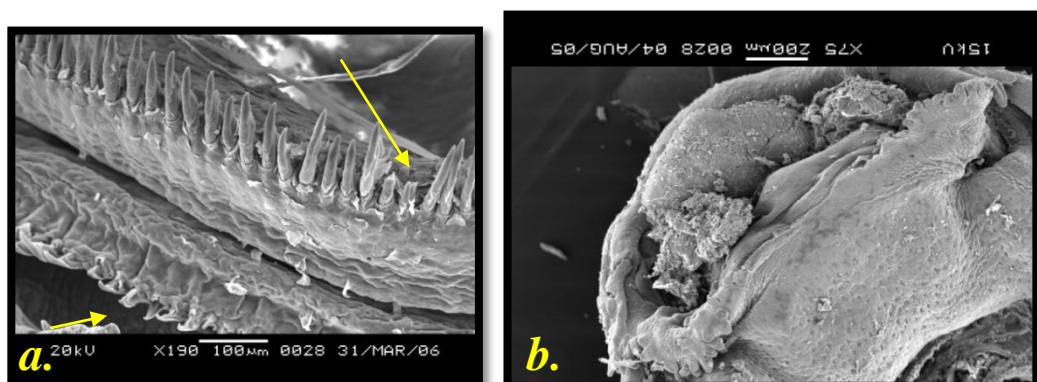


Fig. 4.95(a&b): Scanning electron micrographs of degenerating (a.) posterior tooth rows of *Euphlyctis cyanophlyctis* at stage 40, and (b.) degenerating oral structures of *Euphlyctis cyanophlyctis* at stage 43.

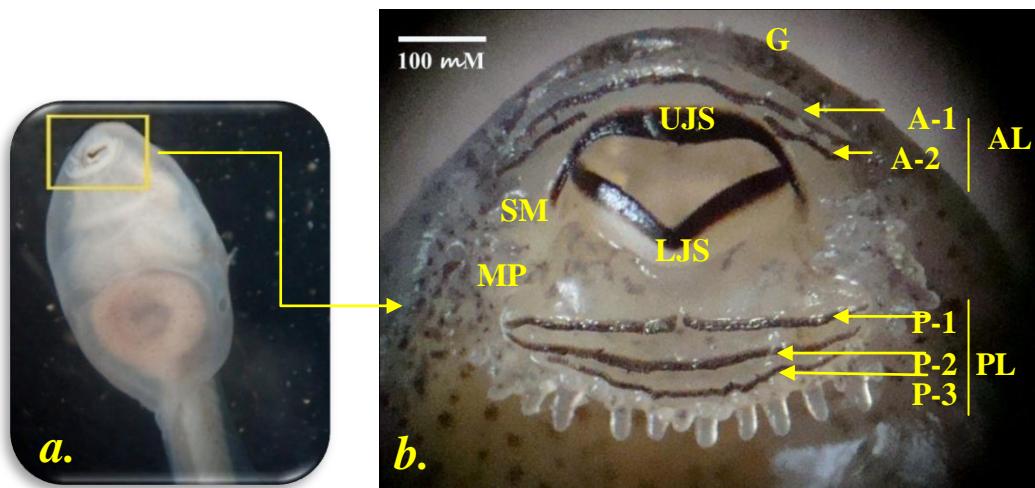


Fig.4.96 (a&b): Light micrographs of stage-25 of *Hylarana nicobariensis* (a.) ventral view and (b.) oral apparatus. LTRF (Labial Teeth Row Formula) = 2(2)/3(1)

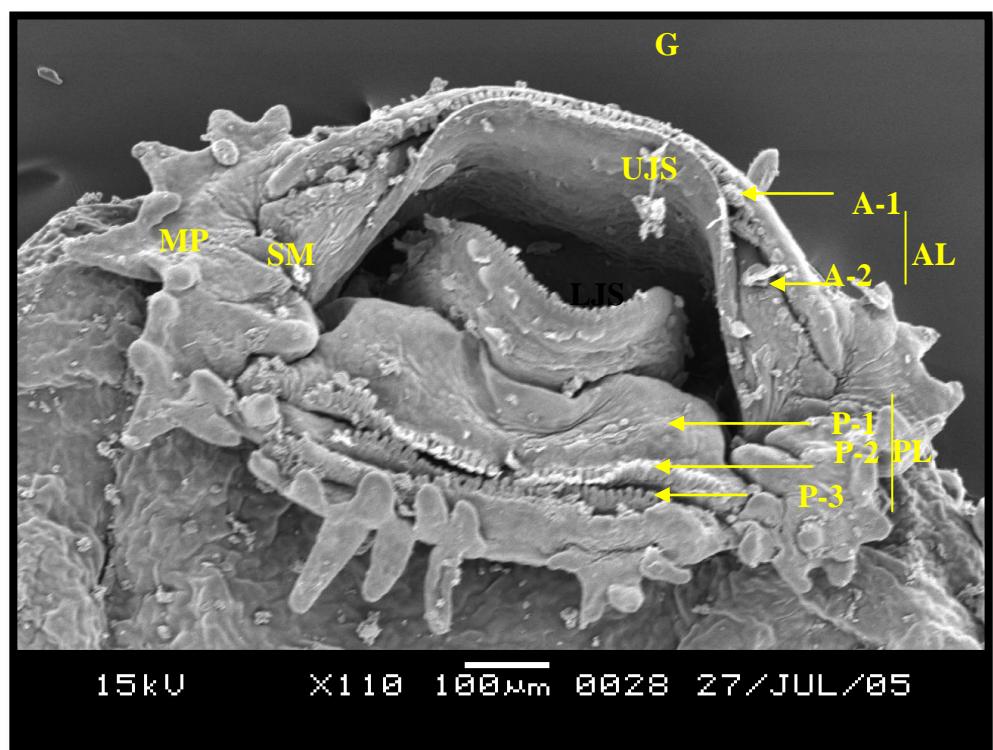


Fig. 4.97: Scanning electron micrograph of oral apparatus at stage 25 of *Hylarana nicobariensis*, A-1: Anterior tooth row 1; P-1: Posterior tooth row 1; P-2: Posterior tooth row; AL: Anterior labium; PL: Posterior labium; SM: Submarginal papillae; G: Dorsal gap.

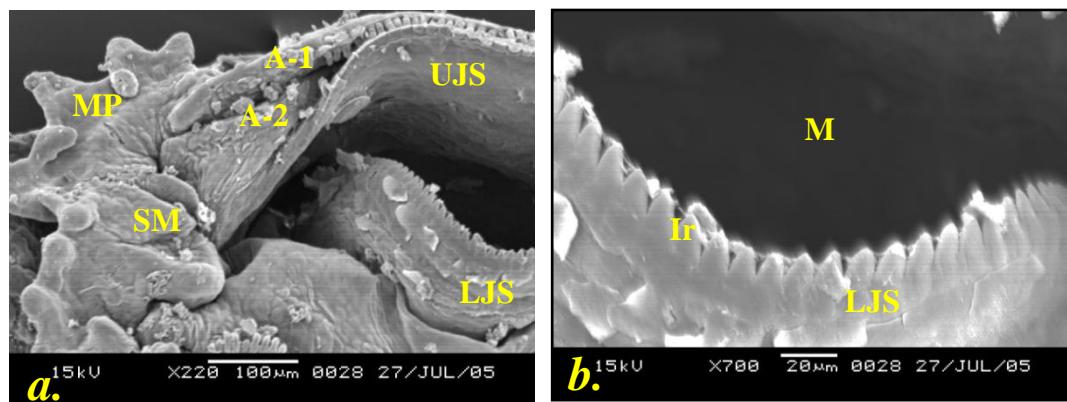


Fig.4.98(a&b): Scanning electron micrographs of *Hylarana nicobariensis* at stage 25. (a.) showing A-1, A-2, MP, SM, UJS & LJS, and (b.) close up view of LJS showing mouth (M) and infrarostrodont (Ir)

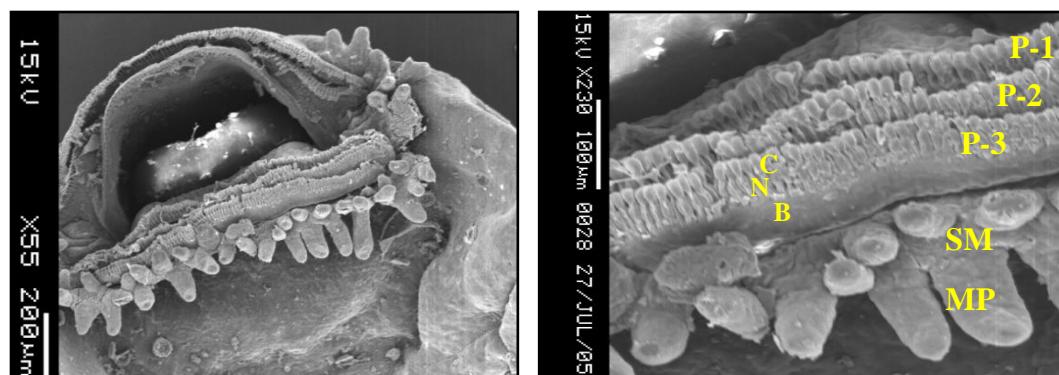


Fig. 4.99(a&b): Scanning electron micrographs of (a.) whole oral apparatus, and (b.) close up view of posterior labium showing P-1, P-2, P-3, SM and MP at stage 29 of *Hylarana nicobariensis*. B: Base; N: Neck; C: Cusp.

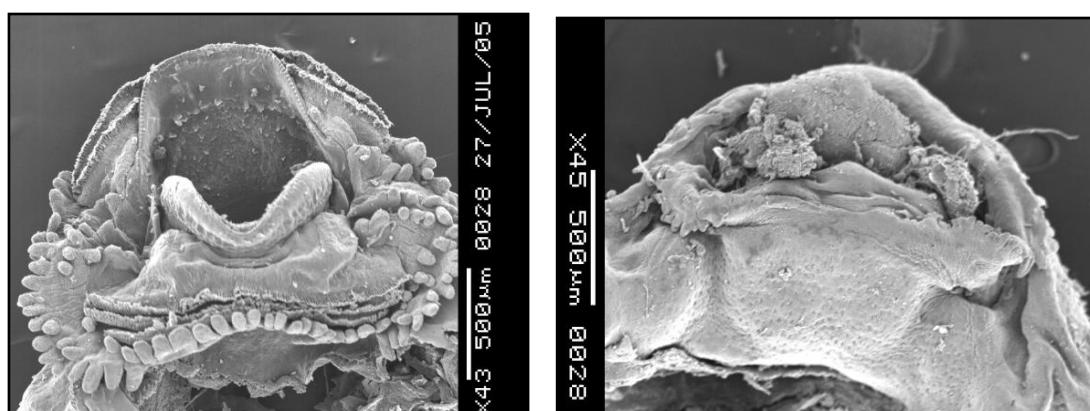


Fig. 4.100(a&b): Scanning electron micrographs of *Hylarana nicobariensis* (a.) whole oral apparatus at stage 41, and (b.) degenerating oral apparatus at stage 42.

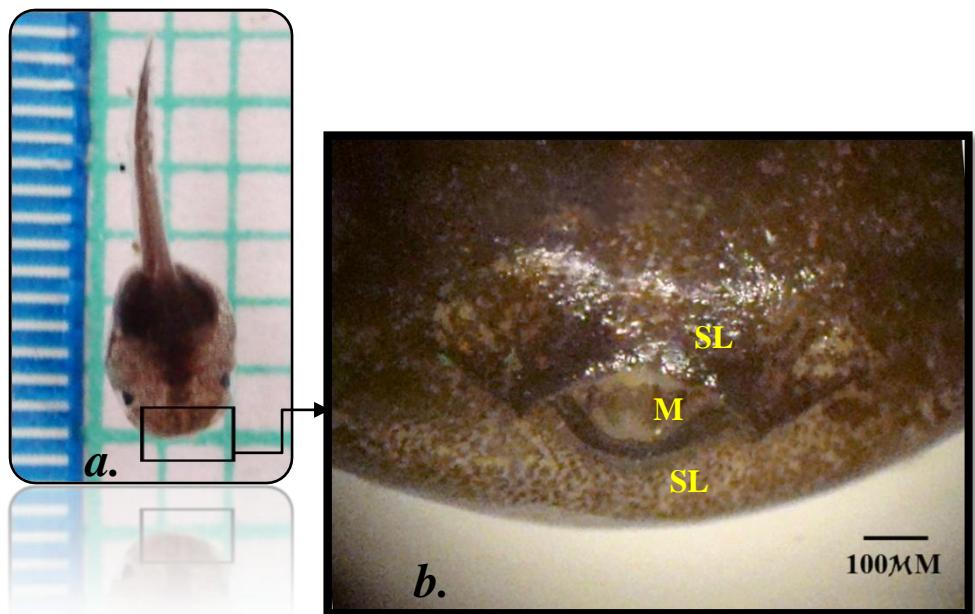


Fig. 4.101(a&b): Light micrographs of stage-25 of *Kaloula pulchra* (a.) dorsal view of whole body, and (b.) oral apparatus. SL: Semicircular labial flap; M: Mouth.

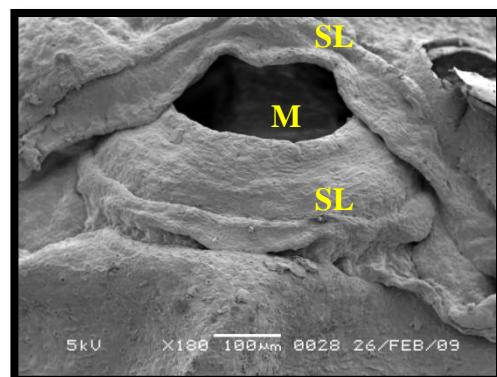


Fig. 4.102: Scanning electron micrograph of oral apparatus at stage 25 of *Kaloula pulchra*.



Fig. 4.103: Scanning electron micrograph of degenerating oral apparatus at stage 42 of *Kaloula pulchra*.

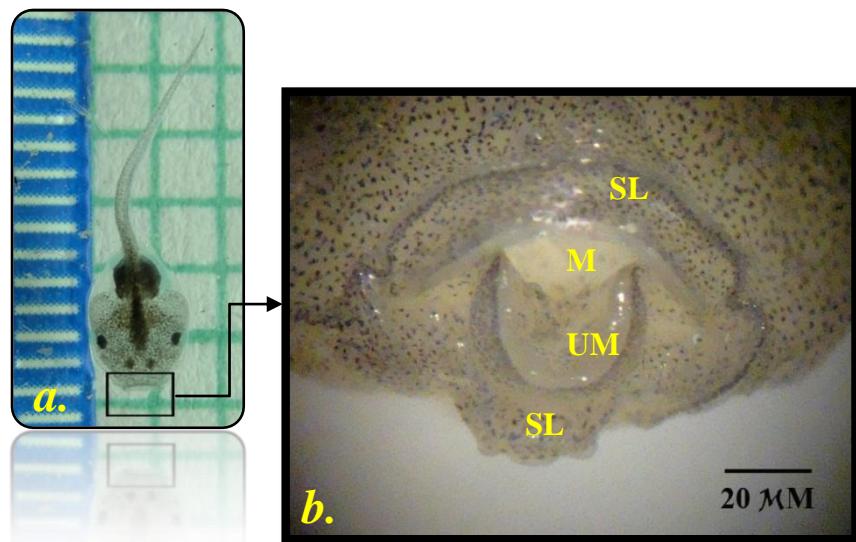


Fig. 4.104: Light micrographs of stage-25 of *Microhyla berdmorei* (a.) dorsal view of whole body, and (b.) oral apparatus. SL: Semicircular labial flap; M: Mouth; UM: U-shaped Medial notch.

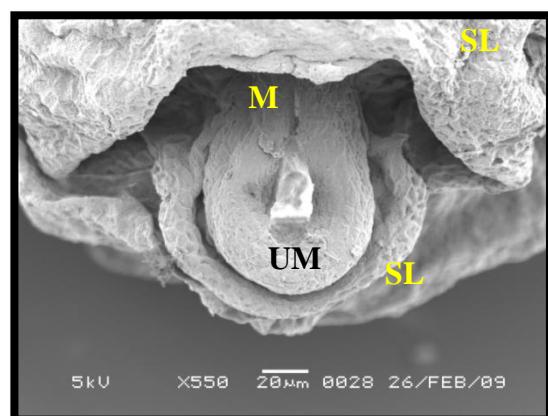


Fig. 4.105: Scanning electron micrograph of oral apparatus at stage 25 of *Microhyla berdmorei*.

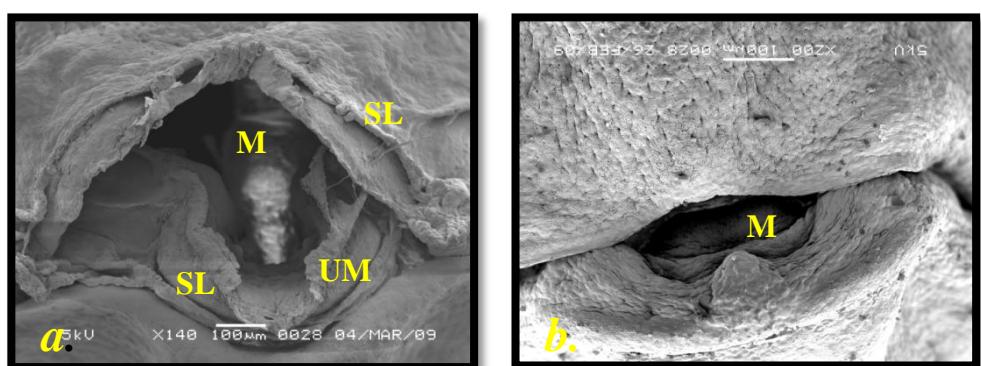


Fig. 4.106(a&b): Scanning electron micrographs of degenerating oral apparatus at (a.) stage 42, and (b.) stage 43 of *Microhyla berdmorei*



Fig. 4.107: Food items found in the gut of adult *Euphlyctis cyanophlyctis*



Fig. 4.108: Food items found in the gut of adult *Hylarana nicobariensis*

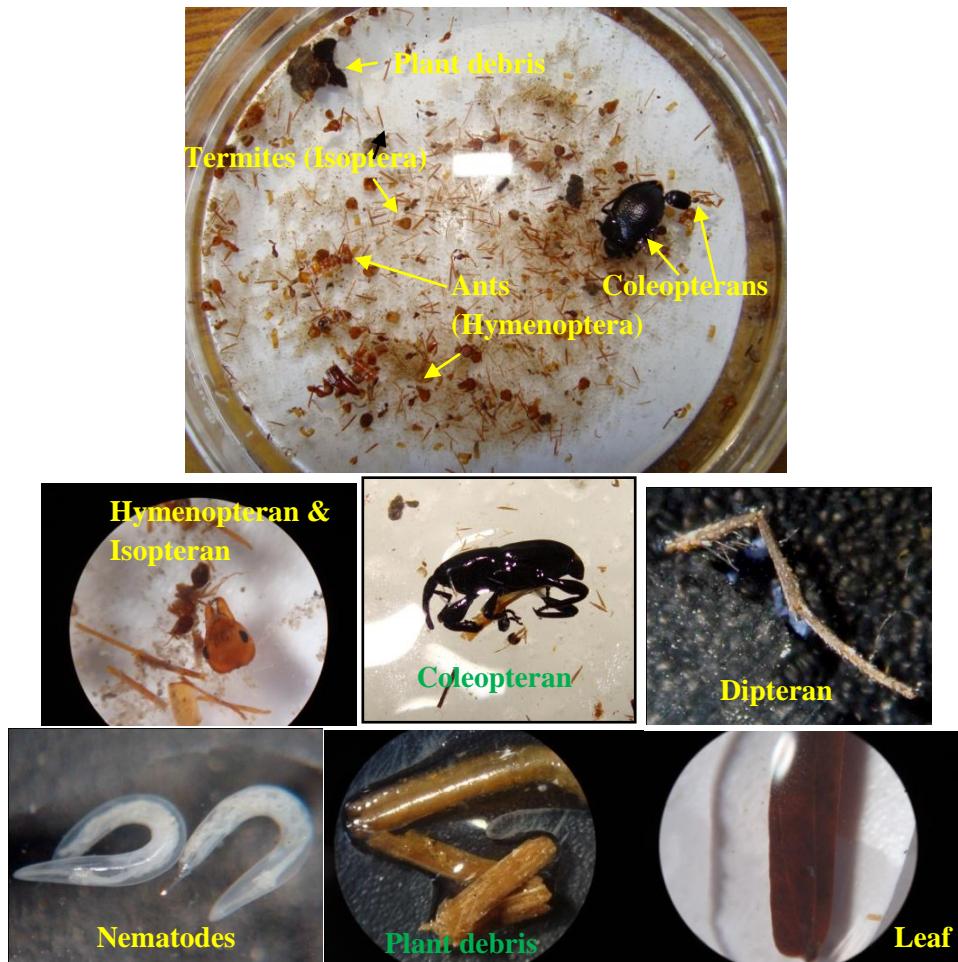


Fig. 4.109: Food items found in the gut of adult *Kaloula pulchra*



Fig. 4.110: Food items found in the gut of adult *Microhyla berdmorei*

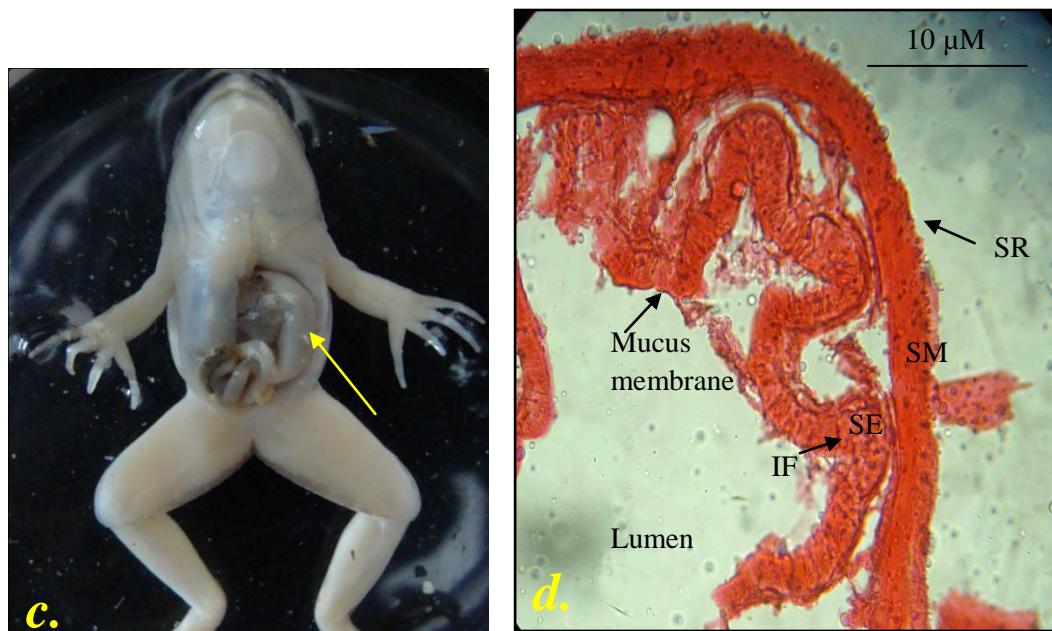
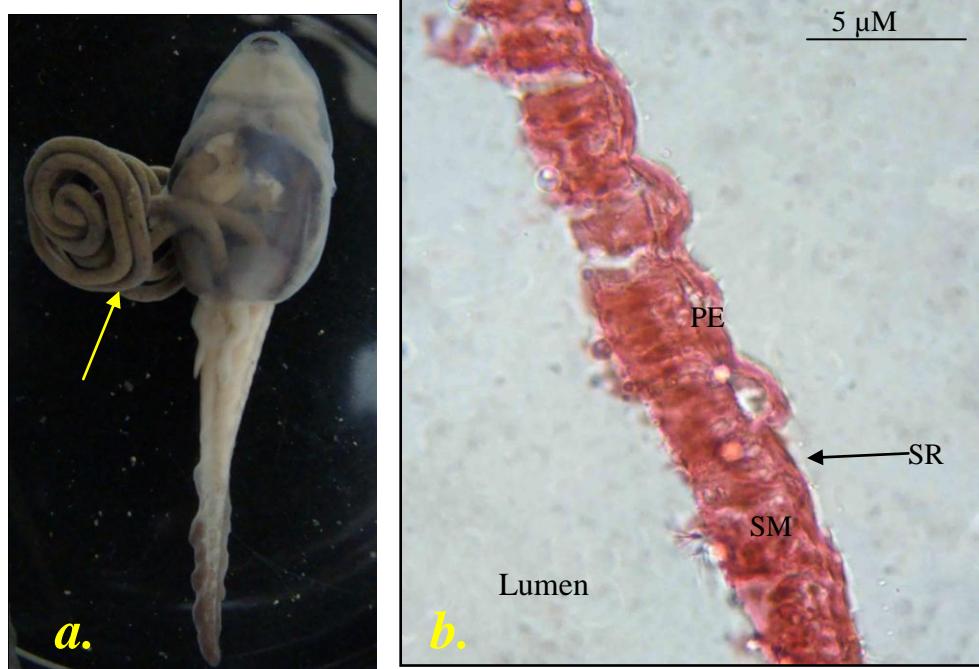


Fig. 4.111(a-d): Remodelling of intestines of *Euphlyctis cyanophlyctis* (a.) intestine of tadpole Gosner stage – 35 and (b.) its section; (c.) intestine of froglet (Gosner stage – 46) and (d.) its section. IF: Intestinal fold; PE: Primary epithelium; SE: Secondary epithelium; SM: Submucosa; SR: Serosa.

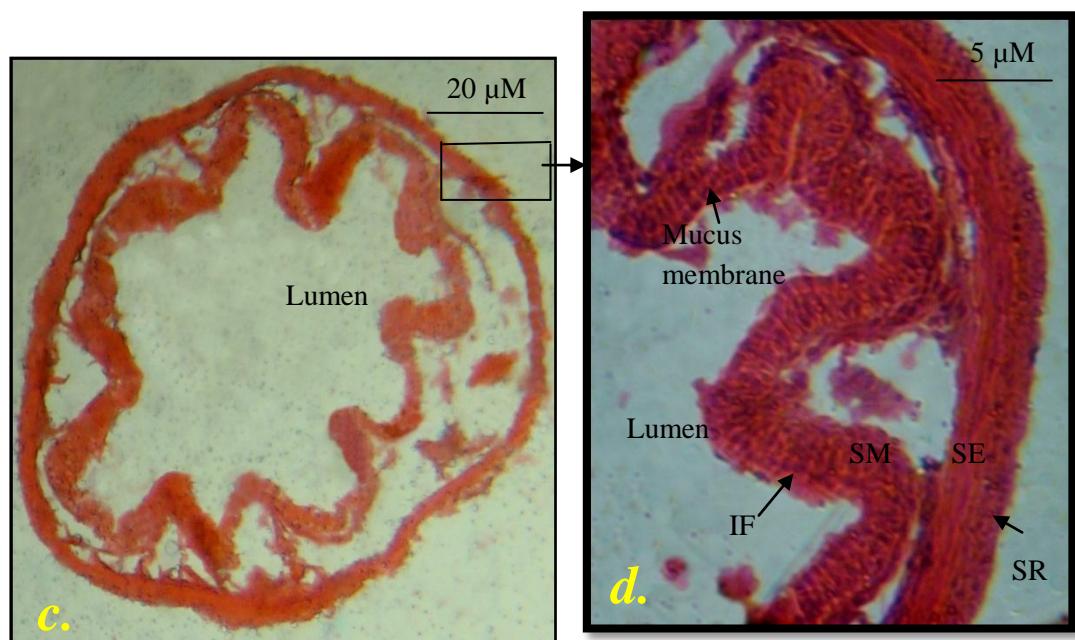
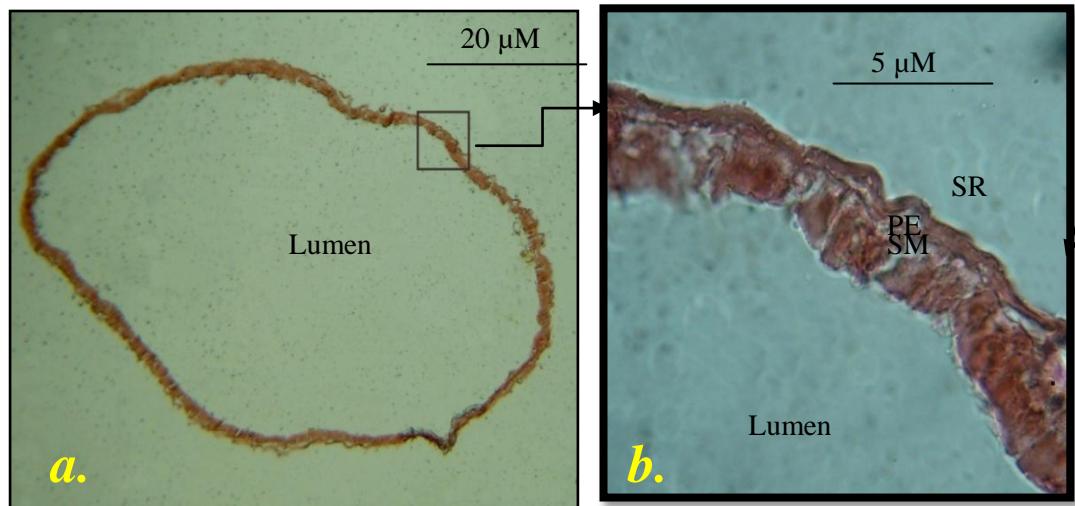


Fig. 4.112(a-d): Remodelling of intestines of *Hylarana nicobariensis* (a.) section of intestine of tadpole Gosner stage – 26 and (b.) its close up view; (c.) section of intestine of froglet (Gosner stage – 46) and (d.) its close up view. IF: Intestinal fold; PE: Primary epithelium; SE: Secondary epithelium; SM: Submucosa; SR: Serosa.

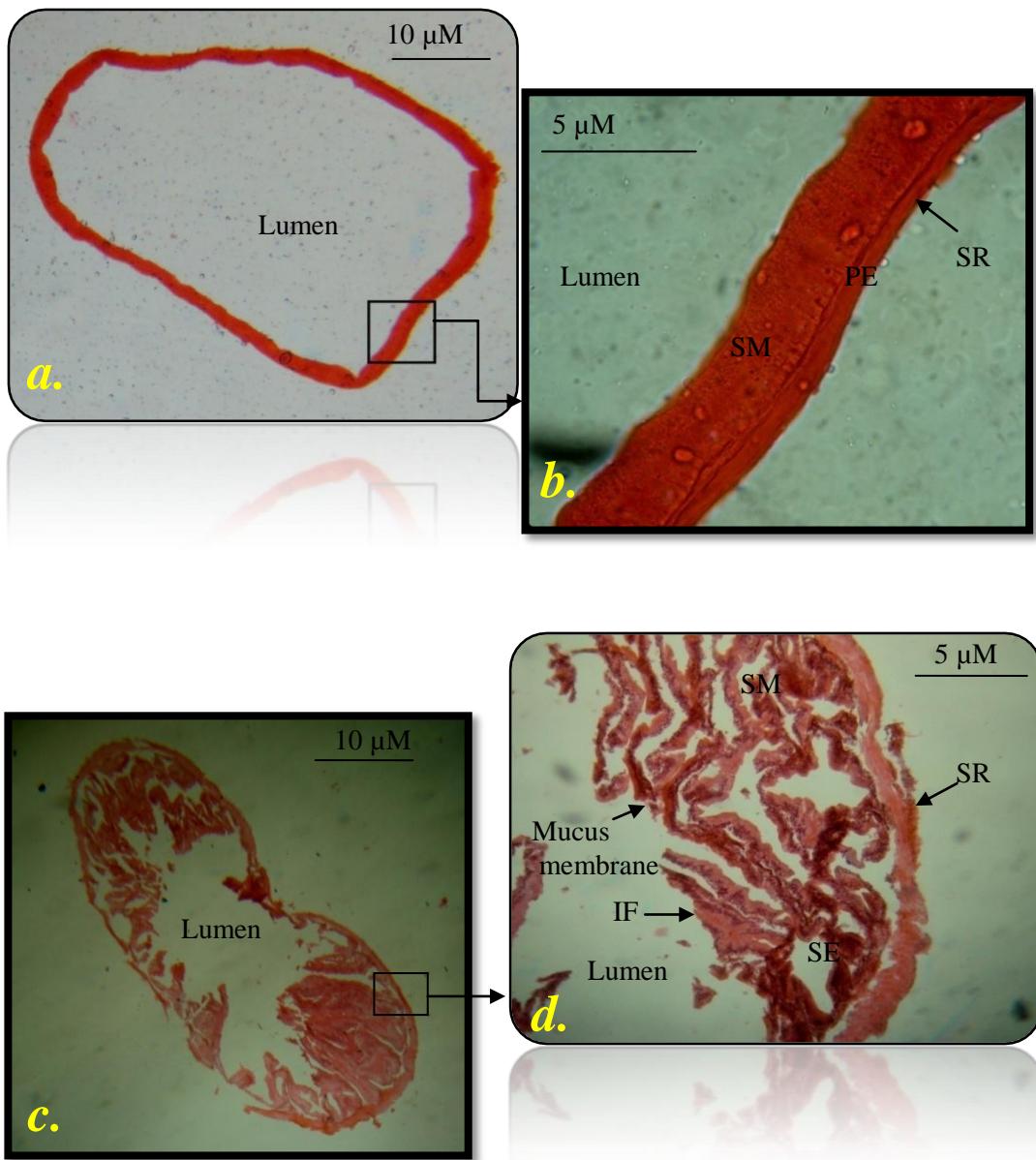


Fig. 4.113(a-d): Remodelling of intestines of *Kaloula pulchra* (a.) section of intestine of tadpole Gosner stage – 32 and (b.) its close up view; (c.) section of intestine of froglet (Gosner stage – 46) and (d.) its close up view. IF: Intestinal fold; PE: Primary epithelium; SE: Secondary epithelium; SM: Submucosa; SR: Serosa.

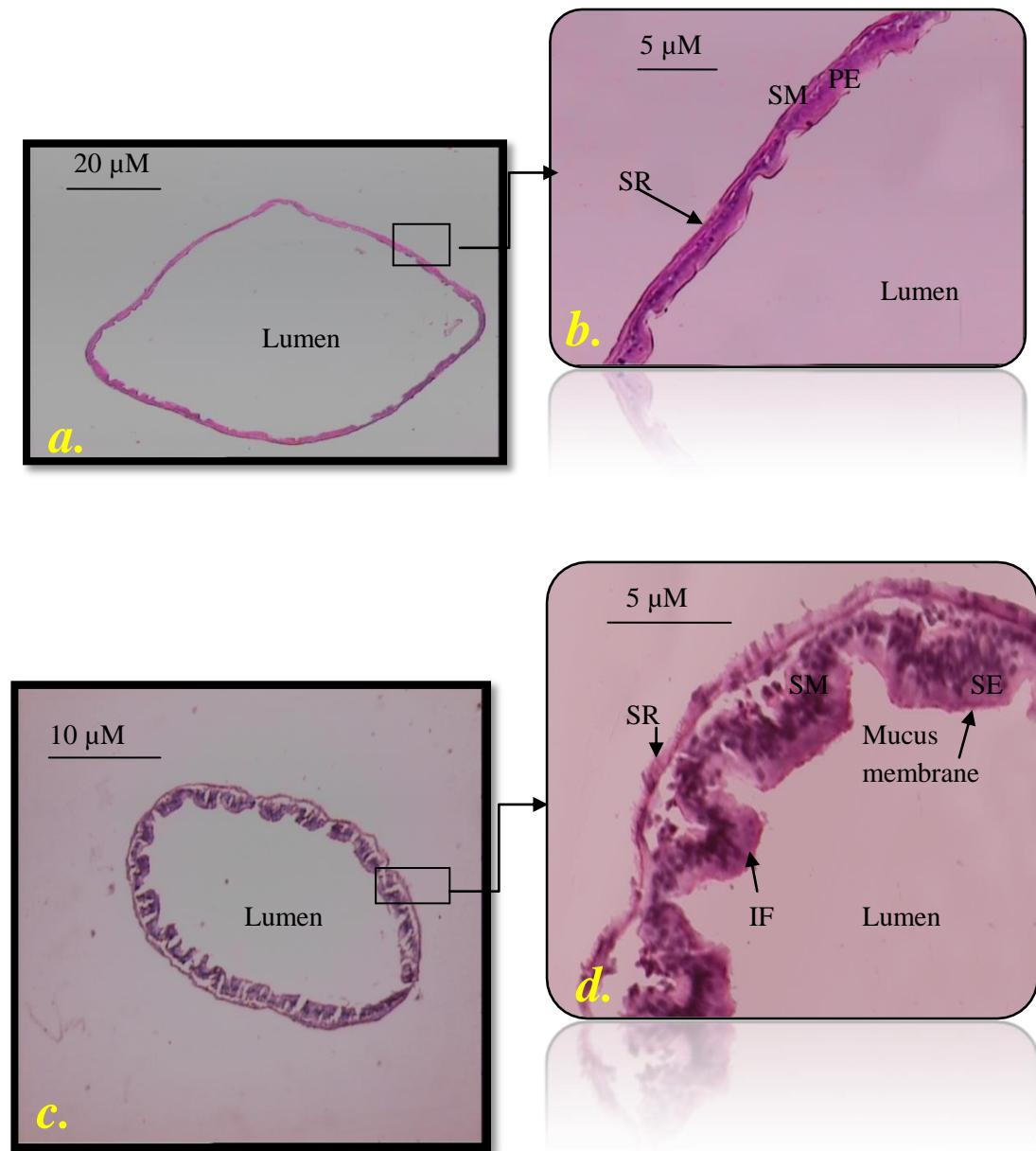


Fig. 4.114 (a-d): Remodelling of intestines of *Microhyla berdmorei* (a.) section of intestine of tadpole Gosner stage – 28 and (b.) its close up view; (c.) section of intestine of froglet (Gosner stage – 46) and (d.) its close up view. IF: Intestinal fold; PE: Primary epithelium; SE: Secondary epithelium; SM: Submucosa; SR: Serosa.

CHAPTER 5

DISCUSSIONS

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I. Distribution of ranid and microhylid anurans prevalent in Mizoram:

Understanding patterns in the abundance and distribution of animals is one of the major goals of ecology (Marsh *et. al.*, 1999). The 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 (Daniels, 1992). The present study revealed the current status on the distribution of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* ranging from very low to high elevated level collected from different parts of all the eight districts of Mizoram.

Chanda (1994) reported *Euphlyctis cyanophlyctis* for the first time from Mizoram and during the present survey, this species was collected from all the thirty surveyed sites throughout all the eight districts from low to high altitudes (46 m – 1460 m asl). However, Chanda (1988) recorded the altitudinal distribution of this species from 500 m – 2500 m in northeast India, and Ao *et. al.* (2003) mentioned that they were widely distributed and available up to 2440 m asl in Nagaland. Ahmed *et. al.* (2009) also reported from the altitude between 40 m – 2500 m. In most of the surveyed areas, *Euphlyctis cyanophlyctis* was available throughout the year and collected from different types of habitat. They were mainly collected from the littoral part of lentic and lotic habitats, water canals and temporary pools of the disturbed area as also mentioned by Inger (1999). Similarly, Chanda (1994) reported that in Meghalaya, *Euphlyctis cyanophlyctis* has been found to occur throughout the

year, in Arunachal Pradesh and Tripura it has been recorded during the breeding season. Outside India, it is also found in Afghanistan, Bangladesh, Iran, Nepal, Pakistan and Sri Lanka.

Hylarana nicobariensis was encountered and collected from a wide variety of habitats, from running waters and their adjacent water bodies, to rice paddy fields, to leaf-litters of forest floors, disturbed environment from the twenty six surveyed sites with the elevation ranging from 46 m to 1460 m asl which agreed with their occurrence between 255 m and 1320 m asl in Padang area of West Sumatra (Inger and Iskandar, 2005). From northeast India, Sen (2004) reported the species from Assam and Tripura only. Outside northeast India, it is also reported from Nicobar Island, Philippines, Thailand, Bali to Peninsular Thailand including Sumatra and Borneo (Frost, 2008). Inger and Stuebing (1989) also reported *Hylarana nicobariensis* as a widely distributed frog of disturbed habitats. The present study reported the presence of *Hylarana nicobariensis* from the state of Mizoram and collection was made during the dry season. In contrast to the other three frogs (*Euphlyctis cyanophlyctis*, *Kaloula pulchra* and *Microhyla berdmorei*) they were rarely encountered among ephemeral pools and puddles which may be located away from the streams or rivers. This species was mainly collected along the streams and rivers during winter season where the current and volume of water is very low. This observation is supported by the occurrence of other stream-breeding frogs near and in the streams during dry season, e.g. *Rana longicrus* and *R. sauteri* in Taiwan (Kuramoto *et. al.*, 1984), *Rana sakuraii* in Japan (Kusano and Hayashi, 2002), etc.

Kaloula pulchra was mostly found to inhabit disturbed areas near human habitation as observed by Inger (1999). *Kaloula pulchra* was first reported from

India by Romer (1949) from Nagaland state, North Eastern India. It was subsequently reported from Tinsukia and Cachar District, Assam state, North Eastern India (Dutta, 1997; Dey *et. al.*, 2000) and also Cherrapunjee, East Khasi Hills District, Meghalaya, (Hooroo *et. al.*, 2002). Outside India, *Kaloula pulchra* is reported from Southern China including Hainan Island, Indochina, Bangladesh, Sumatra, Borneo, and Sulawesi (Indonesia) and Vietnam (Orlov *et. al.*, 2002). Pawar and Birand (2001) reported from three localities, Dampa, Ngengpui and Palak dil of Mizoram. Sailo *et. al.* (2005) reported from only one locality of Mizoram (Sihhmui). However, the present investigation provided more information about the distribution of the species collected from 16 different areas in Mizoram. It thus, seems that *Kaloula pulchra* inhabits various sites of Mizoram irrespective of the altitudes ranging from 46 m – 1460 m asl, indicating that *Kaloula pulchra* has diverse adaptations to live in low and high altitudes. In Vietnam, Orlov *et. al.* (2002) also reported from seaside lowlands and montane regions, ascending up to 1200 m asl.

Microhyla berdmorei was mainly collected from the streams, rivers and their adjacent water bodies. Earlier, Chanda (1994) reported the presence of *Microhyla berdmorei* only from one place of Mizoram, i.e. Serchhip. More information from the present study about the distribution of this species as it was collected from twenty one surveyed sites. This observation on the altitudinal distribution of *Microhyla berdmorei* from 46 m asl to 1460 m asl agreed with the investigation carried out by Chanda (1988). Chanda (1994) reported *Microhyla berdmorei* from different states of India such as Assam, Arunachal Pradesh, Meghalaya and Mizoram. However, Sen (2004) reported the species from Assam, Arunachal Pradesh, Meghalaya, Mizoram and Tripura. Outside India, *Microhyla berdmorei* is

also found in Bangladesh, Cambodia, China, Indonesia, Lao People's Democratic Republic, Malaysia, Myanmar, Thailand and Vietnam (IUCN *et. al.*, 2004).

The present investigation revealed that both *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis* shows their wide distribution throughout the state from lower to higher elevations with different geographic locations as reported by other workers (Mohanty-Hejmadi and Dutta, 1979; Mallick, 1988). This might be due to non-specific in their choice of microhabitat. Das (2008) recorded *Euphlyctis cyanophlyctis* from all kinds of water bodies (ponds, rivers, forest streams, temporary water pool, village water holes and wells, and also swampy areas), 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). Inger (1999) included *Kaloula pulchra*, *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis* among a few species that seem to be most common in severely disturbed environments in southern Asia, where they form large populations and rarely seen in forests. Furthermore, most of these species have extremely large geographic distributions. According to Levins (1968), abundant species tend to have broader niches than rarer ones. Ultsch *et. al.* (1999) also reported that anurans possess physiological, behavioural and morphological plasticity that enables them to survive in many unusual habitats such as arid environments, high elevations, and arboreal pools. Heterogeneous habitats also favour an increase in species richness, since a higher combination of microhabitat types and ecological niches is available (MacArthur, 1968).

It was found that although both *Hylarana nicobariensis* and *Microhyla berdmorei* were usually encountered in the same ecosystem of running water during

winter, these two species shows difference in the microhabitat. While most of *Hylarana nicobariensis* were seen among the main water bodies and the few individuals observed were perched on the ground, logs or rocks within the margins of water course, among the leaf litters, usually close to riffles or slow-flowing, the latter occupied the shaded littoral region or perched either on the ground or on the muddy banks of streams near slow-flowing or lentic pool sections. It may be suggested that the choice of specific microhabitats is related to morphological, physiological and behavioural adaptations of species as reported by Crump (1971), Pough *et. al.* (1977) and Cardoso *et. al.* (1989). Spatial or/and temporal partitioning of reproductive resources are thought to be important mechanisms enabling syntopic taxa to avoid competition (Crump, 1971; Donnelly and Guyer, 1994; Maiorana 1976). Many factors have been suggested to influence patterns of species distribution and assemblage composition at specific sites, such as competition (Morin, 1983), predation (Gascon, 1991a; Eterovick and Sazima, 2000), morphological and behavioural attributes (Crump, 1974; Toft, 1985).

The field survey and collections revealed that *Kaloula pulchra* and *Microhyla berdmorei* preferred ephemeral pools and backwaters of stream or river with some vegetation surrounding it as their habitat. These temporary pools and water holes which are formed during the monsoon and backwaters flanks with vegetation provides excellent habitat for breeding and development of these microhylid frogs. Zug *et. al.* (1998) observed *Kaloula pulchra* predominantly in the forest, and all breeding males in forest or forest-edge pools, although it is a known resident of garden and landscaped sites in villages and towns. Das (2008) reported that although *Kaloula pulchra* is a burrowing frog, the individuals of the species

climbs well and often seen 30 cm– 1 m above surface and individuals were recorded from tree hole at 1 - 2 m above. Inger *et. al.* (1999) recorded *Microhyla berdmorei* from forest floor and on banks of forest streams in litter while surveying the frog fauna of Vietnam. Werner and Glennemeier (1999) also mentioned that canopy cover has been shown to be an important gradient influencing the distribution of anuran species. The present observation suggested that these two microhylids select a shaded or damp microhabitat which is also supported by the burrowing behaviour of *Kaloula pulchra* inside the dark and cool subterranean.

Amphibian diversity is sensitive to a number of environment characteristics. Environmental variables are known to influence species distribution in many ways (e.g. Gascon, 1991a; Leuven *et. al.*, 1986). Species composition and/or species richness of tropical and temperate zone amphibian communities change over gradients in precipitation (Caughey and Gall, 1985; Duellman, 1988; Owen and Dixon, 1989; Lee, 1993), soil moisture (Friend and Cellier, 1990; Woinarski and Gambold, 1992), altitude (Schimdt, 1936; Hairston, 1949; Rivero and Mayorga, 1963; Lynch and Duellman, 1980; Fauth *et. al.*, 1989), forest type (Crump, 1971; Inger and Colwell, 1977), forest structure (Lieberman, 1986; Gascon, 1991a) and, ecological and historical factors (Angermeier and Schlosser, 1989; Eterovick and Fernandes, 2001). Moisture significantly influences amphibians during different stages of their life cycle and is an important abiotic factor influencing the assemblage (Duellman and Trueb, 1994; Heatwole, 1974). Phylogeny may also have an influence in some cases, while in others environmental factors may be more important in determining species distribution (Eterovick and Fernandes, 2001). 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). Since altitude of a place influence on other climatic factors, so these four species of frogs *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra*, and *Microhyla berdmorei* are expected to exhibit a great degree of variation in their behavior, physiology, morphology and distribution. In tropical regions, forest fragmentation is an important result of human activity and might influence amphibian assemblages through edge effects on environmental parameters (Ranney, 1977; Ranney *et. al.*, 1981; Lovejoy *et. al.*, 1986; Malcolm, 1994). The composition of tropical amphibian assemblages is correlated with natural environmental gradients and amphibian communities vary on a gross scale depending on the presence of human activities (Schimdt, 1936; Crump, 1971; Inger and Colwell, 1977; Duellman, 1978). Pearman (1997) also reported that the composition of tropical amphibian assemblages was associated with environmental variation that accompanies forest intervention and the response of tropical amphibian assemblages to anthropogenic variation is complex, as witnessed by conflicts in the literature.

This present observation supported that amphibian abundance was strongly influenced by the presence of water. Sites with deeper ponds that dried later in the summer supported more amphibians than shallower ponds that dried early in the summer. Interestingly, frog abundance was significantly related to the distance to the nearest water sources. On the basis of the present investigations, it may be suggested that the distribution of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra*, and *Microhyla berdmorei* in different parts of Mizoram is due to regional and local factors influenced by topography, geographic location, humidity, seasonal distribution of rainfall, moisture, temperature, vegetation and physiological

tolerances of the species. This investigation provides more information about the distribution of these four species in different parts of Mizoram. Moreover, these species are even adapted to disturbed environments. Such investigations need to be accomplished in the very near future because each year witnesses more destruction of the natural environment.

Morphometric measurements and Sexual dimorphism:

The Snout-vent length (SVL) of *Euphlyctis cyanophlyctis* from the present record i. e., males ranges from 32.20 mm - 46.80 mm and females 31.23 mm - 61.15 mm agreed with SVL of specimens collected from Nepal: 40 mm – 60 mm (Mitchell and Zug, 1986), males = 30.5 mm – 38.0 mm and females = 41.5 mm – 57.5 mm (Schleich and Kästle, 2002); SVL of specimens collected from Nagaland: males = 38 mm - 66 mm and females = 45 mm - 92 mm (Ao *et. al.*, 2003) and, 65 mm (Chanda, 2002; Ahmed *et. al.* 2009). Males are usually smaller in size than female, and vocal slits are present in male. During the breeding season, males are usually with a weakly developed nuptial pad at the base of the first finger as reported by Dutta and Manamendra-Arachchi (1996). The venter in female is usually marbled while it is white in male as also reported by Schleich and Kästle (2002).

The present measurement on the snout-vent length (SVL) of *Hylarana nicobariensis*, males 32.34 mm - 48.48 mm (mean = 38.04 ± 0.70 mm) and females 42.36 mm - 58.6 mm (mean = 49.72 ± 0.73 mm) accord with 55 mm (Taylor, 1962; Chanda, 2002), 47 mm – 52 mm (Iskandar, 2004). Males were smaller than females as it was observed among other anurans by other workers (e.g. Kam *et. al.*, 1998; Gilliespie *et. al.*, 2004).

From the present study, Snout-vent length (SVL) of *Kaloula pulchra* males ranges from 57.74 mm - 69.76 mm (mean= 61.92 ± 0.49) and females 59.90 - 69.94 mm (mean= 64.60 ± 0.51) which is more or less similar to those of Western China with SVL = 69 mm (Liu, 1950), Thailand specimens: male SVL = 65 mm and female SVL = 70mm (Taylor 1962); 61 mm – 70 mm from Chatthin Wildlife Sanctuary, Myanmar (Zug *et. al.*, 1998); 80 mm – 85 mm (Chanda, 2002), male: 67.3 mm from the Western Ghats described by Kanamadi *et. al.*, (2002), male: 55 mm and female: 58 mm from Meghalaya (Hooroo *et. al.*, 2002); male: 56 mm – 64 mm and female: 69 mm from Sihhmui, Mizoram (Sailo *et. al.*, 2005) and 85 mm by Ahmed *et. al.* (2009). Males have darker throats than females and females generally being larger than males.

No distinct sexual dimorphism was observed in *Microhyla berdmorei* except the females were slightly larger (Mean \pm SE = 33.9 ± 0.16 ; range = 31.02 mm - 34.35 mm; n = 60) than the males (Mean \pm SE = 31.03 ± 0.17 ; range = 28.45 mm - 33.88 mm; n = 60), and males are usually with darker venter and strong fold across neck as also reported by Taylor (1962). Inger *et. al.* (1999) recorded *Microhyla berdmorei* female SVL range= 36.9 mm - 41.6 mm (Mean \pm SE= 38.96 ± 0.70) and male SVL range= 30.9 mm - 38.2 mm (Mean \pm SE= 35.15 ± 0.68). Chanda (2002) also recorded the average SVL of *Microhyla berdmorei* as 45 mm.

From the present investigation it was found that there was a minor variation on the body size within the same species from different geographical areas. Avise (2000) reported that geographically structured variation in morphology, particularly body size, is common within many species. The minor differences in the snout-vent length was probably due to the geographical variation that influences difference in

density, nutrients and temperature as all these parameters affect growth (Richards, 1958; Rugh, 1962). However, the causes and maintenance of geographical variation in morphology (including body size) are likely to be complex, and are not always well understood (Case and Schwaner, 1993; Partridge and French, 1996; Baez and Brown, 1997; Malhotra and Thorpe, 1997). Many macro- and micro-evolutionary processes, both adaptive and non-adaptive, have been proposed for the maintenance of variation in body size and shape. Examples of potential forces producing geographical variability in morphology include: (i) selection in response to geographical variation in prey type or predation pressure (e.g. Shine, 1987; Arnett and Gotelli, 1999; Schneider *et. al.*, 1999); (ii) effects of climate or other environmental parameters on growth rates (e.g. Riha and Berven, 1991; Castellano *et. al.*, 2000; see also Van Der Have and De Jong, 1996); (iii) variation in the level and nature of sexual selection and sexual size dimorphism (e.g. Endler and Houde, 1995; Wiens *et. al.*, 1999; Storz *et. al.*, 2001); and (iv) non-selective genetic factors such as drift and founder effects (Baker *et. al.*, 1990; Nussbaum and Wu, 1995; Demetrius, 2000). Schäuble (2004) reported climate and latitude as important explanation for variation in sexual size dimorphism.

II. Breeding behavior, habit and habitat, and ecological factors of the study sites:

(i) *Euphlyctis cyanophlyctis*: From the present study conducted in Mizoram, it was found that the breeding activity in *Euphlyctis cyanophlyctis* takes place in the rain-fed pools, ephemeral shallow backwaters of rivers and streams, littoral regions of ponds and lakes filled with dense aquatic vegetations and their breeding season is

greatly influenced by the rainy season in Mizoram from March to August earlier than other places. Many workers observed the breeding activity of this species during monsoon period, May to October (Lakshman, 1965), April to September (Sarkar and Rao, 1968), June to August (Saidapur and Nadkarni, 1974; Mallick, 1986) and July to September (Pancharatna and Saidapur, 1985). McCann (1932) has indicated about the possibility of the same species breeding at other times under suitable circumstances. Present data on the ANOVA test showed that there was significantly different between breeding season and ecological factors like air temperature (only at study site-I), rainfall, and water temperature, where $p<0.05$. By contrast, no significant relationship was detected between breeding season and other ecological factors like pH of water and relative humidity ($p>0.05$). It was suggested that the breeding activity was mainly influenced by rainfall which further controlled other environmental factors. However, Mohanty-Hejmadi and Dutta (1979) also reported the breeding behaviour and development as typically anuran that took place mainly during monsoons. Khan and Malik (1987b) encountered tadpoles of *Euphlyctis cyanophlyctis* before monsoons (March-April) when sympatric species have not started breeding and after monsoons (late August-early October). In the present observation the males and females went into amplexus at night starting from the month of March, it shows that in Mizoram in response to the early shower of rain their breeding activity might be slightly more advance compared with earlier studies. It was also documented that sometimes the amplexus continued throughout the day as observed by Mohanty-Hejmadi and Dutta (1979) and Mallick (1988). During this investigation, as *Euphlyctis cyanophlyctis* shows their availability in the fields throughout the year, it is evident from this studies that this species does not hibernate

during winter which was supported by the observations made by other workers (e.g., Mohanty-Hejmadi and Dutta, 1979; Saidapur, 1989). Amplexing started with the entry of breeding site by female in response to male vocalization, followed by body contact between male and female initiated by female. Mallick (1988) reported that female of *Rana (Euphlyctis) cyanophlyctis* selects her male mate by audio-visual system. In addition to its acoustic function, the vocal sac also may act as a conspicuous visual signal. Despite of the variety of diverse vocal-sac types, their visual relevance has not yet been explored thoroughly (Hödl, 1996). Compared to acoustic cues, visual signals play a subordinate role in amphibian communication (Hirschmann and Hödl, 2006). This present findings support visual display among this species prior to amplexus. Some anuran species, perform remarkable visual displays in variable social contexts (Haddad and Giaretta, 1999; Harding, 1982; Davison, 1984; Richards and James, 1992; Lindquist and Hetherington, 1996, 1998; Hödl, *et. al.*, 1997; Amézquita and Hödl, 2004). Durant and Doyle (1975) and Wells (1980) reported that diurnal species of anurans commonly produce visual displays. Visual signalling so far has been recorded only in tropical frogs, with many species occurring along streams and creeks (Hödl and Amézquita, 2001; Lindquist and Hetherington, 1998). Mallick (1988) reported that males of *Euphlyctis cyanophlyctis* vocalize in the breeding territory in the temporary pools/ditches with dense vegetations filled with accumulated rain water very near the side of pond/permanent territorial habitat as breeding sites. Khan (1991) also reported that females of *Euphlyctis cyanophlyctis* are known to pair with several males, one after another, laying eggs with each partner.

(ii.) *Hylarana nicobariensis*: The breeding season of *Hylarana nicobariensis* coincides with winter season (October - February) in Mizoram which was also observed among other stream breeders e.g. *Rana longicrus* and *R. sauteri* (Kuramoto *et. al.*, 1984) from Taiwan, *R. sakuraii* from Honshu, Japan (Matsui and Matsui, 1990), *R. swinhoana* (Kam, *et. al.*, 1998), four species of the *Rana narina* complex (Kusano and Hayashi, 2002), *Rana chalconota* and *R. celebensis* (Gilliespie *et. al.*, 2004). ANOVA test on the breeding season against environmental factors like air temperature, relative humidity, rainfall, water temperature and pH of water (except water pH at the study site-IV, where $p>0.05$) differed significantly. From the present investigation it may be suggested that shallow water and low current during non-rainy season supports survival of larvae, and provides more calling sites and oviposition sites. Moreover, the winter season allows the species to escape from competition with those of monsoon breeders. Up to now, detailed information on the seasonal activity and subtropical regions is lacking; however the available data indicate that the stream breeders reproduce in the dry season (Menzies, 1963; Crump, 1974; Zug and Zug, 1979; Jorgensen *et. al.*, 1986; Aichinger, 1987; Gascon, 1991; Kam *et. al.*, 1998). However, it was suggested that environmental variables such as humidity, temperature and photoperiod may determine anuran breeding period as reported by earlier workers (Navas, 1996; Navas and Bevier, 2001; Hatano *et. al.*, 2002). Observation on the axillary amplexus in *Hylarana nicobariensis* at night time was also reported among other stream breeding frogs e.g. *Rana longicrus* and *R. sauteri* in Taiwan (Kuramoto, 1984).

(iii.) *Kaloula pulchra*: It was observed that reproductively ripe *Kaloula pulchra* can apparently remain quiescent throughout dry periods, beginning spawning activity

only after the first shower of monsoon (late February or March) that makes a wetting condition of the general area. From the present data, ANOVA test on the breeding season against environmental factors (air temperature, relative humidity, rainfall, water temperature and pH) did not differ significantly, where $p>0.05$. It was suggested that only the onset of monsoon stimulates the animals to emerge from their subterranean retreats and strong choruses of breeding aggregations have been heard following prolonged non-violent rains which lasted several days. Prolonged droughts may completely prevent breeding and several continuous days with small rainfall may be as important in the breeding of amphibians as is a single day with heavy rainfall (Berry, 1964). Heyer (1973), Aichinger (1987), Gascon (1991b), and Donnelly and Guyer (1994) suggested that the single physical factor of rainfall distribution regulates anuran reproductive patterns in tropical areas characterized by a pronounced dry season. In Singapore, Berry (1964) also observed that the aggregation of *Kaloula pulchra* at their breeding grounds depended entirely on rain. The breeding period of *Kaloula pulchra* in Mizoram is early to mid-monsoon (late February to May) which agreed with the breeding period of *Kaloula pulchra* in tropical Thailand (Heyer, 1973) and in the Western Ghats (Kanamadi *et. al.*, 2002), more or less similar with other tropical microhylids observed by other workers, e.g., *Uperodon systema* during monsoon (Mohanty-Hejmadi *et. al.*, 1979b), *Ramanella variegata* from June to August (Dutta *et. al.*, 1990-91), *Microhyla rubra* and *Ramanella montana* from May to September in the Western Ghats which receives monsoon during this period (Kanamadi *et. al.*, 1994 and Krishna *et. al.*, 2004), but quite different from the pattern of continuous rainfall-dependent breeding in *Kaloula pulchra* and other microhylids reported from temperate region where climatic

condition is not seasonal as reported by Berry (1964). The present observation on the breeding sites in small pools, usually seasonal rain pools, or ponds was supported by other reports (Leong and Chou, 1999; Kuangyang *et. al.*, 2004). Observation on the males calling in the water at the edge of pools was also reported by Zug *et. al.* (1998), and the axillary amplexus was like those of other microhylids (Leong and Chou, 1999; Heying, 2003).

(iv.) *Microhyla berdmorei* : The present results indicate that the breeding activity of *Microhyla berdmorei* in Mizoram starts from October with the decreased in rainfall followed by prolonged dry season. The breeding period of *Microhyla berdmorei* during non-rainy season/winter (October-March) in Mizoram was strongly supported by the observation made by Heyer (1973) in Thailand, but in contrast with other microhylids like, *Kaloula pulchra*, *Microhyla butleri* and *Microhyla heymonsi* (Berry, 1964), *Microhyla ornata* (Mohanty-Hejmadi *et. al.*, 1980), *Microhyla rubra* (Kanamadi *et. al.*, 1994), *Ramanella variegata* (Dutta *et. al.*, 1990-91) and *Uperodon systoma* (Mohanty-Hejmadi *et. al.*, 1979b), that breeds during rainy/monsoon season. Results on the ANOVA test showed that the breeding season against ecological factors like air temperature, relative humidity, rainfall and water temperature differed significantly, where $p<0.05$. But the breeding season against pH of water did not differ significantly, where $p>0.05$. It was suggested that since *Microhyla berdmorei* is a stream breeder unlike other microhylids mentioned above, this species begins to breed during the initial part of dry season until the next monsoon has come when no more floods occur in the permanent stream and river. The present observation on the breeding that takes place in still pools was also reported by Van Dijk *et. al.* (2004). This present observation on the axillary

amplexus was also observed among other microhylids like, *Microhyla rubra* (Kanamadi *et. al.*, 1994), *Ramanella montana* (Krishna *et. al.*, 2004) etc.

From the present investigations, it was found that the timing of the reproductive activities of *Euphlyctis cyanophlyctis* and *Kaloula pulchra* was clearly affected by rainfall. Since amphibians are poikilothermic and also that the majority of them depend upon water for breeding, their reproductive activities are greatly affected by the changing climatic factors such as the temperature, rainfall, day light length and relative humidity. This factor may indicate water availability, which generally stimulates anuran reproduction (Telford and Dyson, 1990; Gascon, 1991). Gopalakrishnan and Rajasekarasetty (1978) reported that studies on the annual reproductive behavior of the temperate, subtropical and tropical species of Amphibia indicate that the internal hypophysio-gonadal rhythm is under the mark influence of a variety of external factors. A complex pattern of the external stimuli is required to induce the breeding behavior, no single stimulus is adequate and yet temperature and rainfall are important factors to reckon (Amoroso and Marshall, 1960). Explanations of temporal variation in breeding activity in anurans often implicate rainfall because of the predominant external mode of fertilization, and the aquatic larval phase generally makes the availability of free water an essential requirement for breeding (Duellman and Trueb, 1994). Because anurans are ectotherms, temperature also may be important (Byrne, 2002).

In the present study, the breeding season of both stream breeders *Hylarana nicobariensis* and *Microhyla berdmorei* coincides with winter season in Mizoram. The occurrence of the tadpoles of *Hylarana nicobariensis* and *Microhyla berdmorei* in the same habitat from Peninsular Malaysia was also reported by Ming (2005). An

interesting finding is that the breeding peaks of *Hylarana nicobariensis* occurred during December, when the water become very shallow with minimum velocity, when most frogs were relatively inactive. Results on the ANOVA test showed that breeding season of *Hylarana nicobariensis* and *Microhyla berdmorei*, against environmental factors (air temperature, relative humidity, rainfall, and water temperature, where $p<0.05$) differed significantly, but there was no significant relationship between breeding season and pH of water ($p>0.05$). Heyer (1973), Tsuji and Kawamichi (1996) also mentioned that many riverine frog communities in seasonal environments have relatively synchronous breeding seasons, possibly reflecting abiotic constraints on timing of breeding, such as seasonal drying or flooding. Kam *et. al.* (1998) suggested that stream breeders may have a different reproductive phenology from ephemeral pond breeders, because water is readily available throughout the larval period and dessication is never a threat to the larvae that live in the water. Fukuyama and Kusano (1992) also reported that floods during wet season reduce the breeding activity of a riparian frog, *Buergeria buergeri*, by reducing the suitable calling sites, such as emerged stones in the flowing riffles. This study also suggested that floods not only reduce suitable calling sites, such as emerged stones in the flowing but also causes larval mortality as observed by other workers (Metter, 1968; Zug and Zug, 1979; Petranka, 1984; Petranka and Sih, 1986; Fukayamo and Kusano, 1992; Kam *et. al.*, 1998). The results on these two species support the hypothesis that the stream breeders have been known to select dry season for breeding because of the low water flowing during that time which ensure better survival of their offspring as observed by other workers (Metter, 1968; Zug and Zug, 1979; Petranka, 1984; Petranka and Sih, 1986; Kam *et. al.*, 1998).

Other than *Euphlyctis cyanophlyctis*, no sign of visual cues was observed among the other three species. Lindquist and Hetherington (1998), and Hödl and Amézquita (2001) reported that the evolution of visual communication in anuran environments, and highly structured habitats. During the breeding season, the breeding areas of *Euphlyctis cyanophlyctis* (at study sites I and IV) are also utilised by another breeding frogs like *Duttaphrynus melanostictus*, *Fejervarya limnocharis*, *Hoplobatrachus tigerina*, *Polypedates leucomystax* and *Hylarana leptoglossa* that turns the breeding ground into a quite noisy environment. While *Kaloula pulchra* breeds in solitary breeding sites, mis-amplexus between male *Hylarana nicobarensis* and its sympatric female *Microhyla berdmorei* was occasionally encountered in the fields during their breeding season. The present observation reported that unlike *Hylarana nicobariensis*, the other three species i.e. *Euphlyctis cyanophlyctis*, *Kaloula pulchra* and *Microhyla berdmorei* avoid the mainstream by retiring to the ephemeral pools and puddles along the course of water channel. It is suggested that choice of breeding sites by amphibians can be influenced by the quality of the surrounding terrestrial habitat as well as by characteristics of the water body (Alford, 1999; Semlitsch and Bodie, 2003).

Courtship and mating calls: The mating call of *Euphlyctis cyanophlyctis* lasted for about 599.7 ms emitted at an interval of 1.302 ms, each call is composed of 5 - 12 pulses, and the frequency spectra ranged between 1140 – 1609 Hz (Fig. 4.9) agreed with the analysed made by Roy *et. al.*, (1995) with call duration 615 ms, 7 pulses per call and frequency ranged between 1160 – 3900 Hz. It shows a little difference with the observation made by Roy *et.al.* (1998) in which call duration lasted for about

1.122 s with intervals of about 3.574 s, each call having about 9 pulses and dominant frequencies at about 780 Hz - 1420 Hz.

The advertisement calls of *Hylarana nicobariensis* was with a low pitch as observed among other stream-breeding ranids (Bain *et. al.*, 2003, Gilliespie *et. al.*, 2004 etc.) winter breeding ranid, *Rana longicrus* (Kuramoto *et. al.*, 1984), and other ranids like *Hylarana baramica*, *H. glandulosa*, *H. picturata* and *H. luctuosa* as reported by Zainudin *et. al.* (2010). The call consisted of 5 - 9 notes emitted at an interval of 0. 628 - 1.645s, each note ranged from 0.141-0.165 s with a series of 35 to 47 pulses. A single call lasted for about 1.232 – 2.682 s and the frequency spectra ranged between 2109 – 2296 Hz with a dominant band at 2265 Hz. Jehle and Arak (1998) mentioned that a remarkable feature of communication in *Rana* (*Hylarana*) *nicobariensis* is the highly variable advertisement call which shows a 20-fold variation in duration, comprising 1 – 25 notes and in natural choruses, the duration of the advertisement call is inversely related to the distance between a focal male and its closest calling neighbour.

The advertisement call of *Kaloula pulchra* emitted in a series consisted of a single note as also observed by Heyer (1971) and Kanamadi *et. al.* (2002). The notes lasted 450.8-620.3 ms and were composed of a series of 36-45 pulses. The frequency spectra had a dominant band at 1265 Hz. The call duration of *K. pulchra* from Thailand (Heyer, 1971) ranged from 560 to 600 ms, consisted of 18-21 pulses/call, and the frequency spectra had a dominant band at 250 Hz. Whereas the call duration of the same species from Western Ghats varied from 318 to 932 ms with 28-56 pulses/call and dominant frequencies were between 400-1220 Hz. This analysis shows a little difference with others except in the call duration.

No detail data was available on the advertisement call of *Microhyla berdmorei* (Sukumaran *et. al.*, 2006). From the present analysis it is found that the call is species specific and serves to attract conspecific females. The dominant frequency spectra in the present analysis (1677 Hz) agreed with (1500 – 1800 Hz) as reported by Heyer (1971). The call of *Microhyla berdmorei* is very similar to the call of *M. borneensis*. The main difference is the higher dominant frequency (1400 – 2900 Hz) in calls of the latter (Dehling, 2010). The durations of a single call and note (1.4 – 1.6 s and 0.2 s respectively), and 7 – 12 pulses is very near to its sympatric species *Microhyla ornata* (Kuramoto and Joshy, 2006) but different in frequency spectra range (1 – 4 kHz) and dominant frequency band (2 – 3 kHz). The advertisement call of *Microhyla rubra* (Kanamadi *et. al.*, 1994a) differs from that of *Microhyla berdmorei* mainly in its longer call duration (138 ms – 228 ms), more pulses (15 – 21) and wider frequency spectra range (300 – 4150 kHz).

Several studies have shown that social as well as environmental factors influence some call characteristics, such as: dominant frequency, number of pulses, duration, and repetition rate of the note (Wells, 1988; Wilczynski and Ryan, 1999). The differences in the call parameters may reflect geographic variation, as described in *Rana ridibunda* (Schneider, 1973; Nevo and Schneider, 1983; Kuhn and Schneider, 1984; Schneider and Sofianidou, 1985), *Bombina orientalis* (Akef and Schneider, 1985; Schneider *et. al.*, 1986), and *Microhyla ornata* (Hiremath, 1991). In several species, body temperature has a greater effect on call length and rate than body size or condition (Rome *et. al.*, 1992; Howard and Young, 1998; Castellano and Giacoma, 1998; Tarano, 2001). In anurans, spectral call properties, such as dominant or fundamental frequency, are usually negatively correlated with body size

because of morphological constraints on the sound producing apparatus (Martin, 1972). A number of factors have been invoked to explain geographic variation in frog calls including reinforcement (Butlin, 1987; Loftus-Hills and Littlejohn, 1992; Howard and Gregory, 1993), changes in the acoustic environment (Ryan, 1988), or a divergence associated with morphological change over the geographic range of the species (Nevo, 1973). Current evidence suggests that advertisement calls vary between and within populations of the same species (e.g. Ryan *et. al.*, 1996; Gergus *et. al.*, 1997; Smith *et. al.*, 2003). Other studies have found considerable intraspecific variation in the advertisement call of frog species that inhabit broad geographic areas with a range of environmental and climatic conditions (Nevo and Capranica, 1985; Ryan and Wilczynski, 1991; Ryan *et. al.*, 1996; Hasegawa *et. al.*, 1999). Geographic divergence in advertisement call structure can be associated with genetic subdivision (Ryan and Wilczynski, 1991; Ryan *et. al.*, 1996; Castellano *et. al.*, 1998).

Oviposition sites: In this study, *Euphlyctis cyanophlyctis* usually deposited their eggs in the shallow littoral region mixed with aquatic vegetation of both lentic and lotic ecosystems. At the beginning of the breeding season, only larger perennial water bodies are occupied. With the advancing monsoon period, temporary ponds and even smallest bodies of water are invaded. Axillary amplexus of *Euphlyctis cyanophlyctis* results in small batches of floating fertilized eggs that soon sink and attached to submerged vegetation as also observed by other workers (Daniel, 1975; Mohanty-Hejmadi and Dutta, 1979; Khan, 1982; Scleich and Kästle, 2002).

Hylarana nicobariensis deposited egg masses on the substrata (pebbles, rocks, boulders, aquatic plants or wood debris) below the water surface of slow-flowing or still pools in streams and rivers. It may be suggested in the present

observation that the eggs are deposited near rocks or boulders, so that the eggs are not washed away by the currents as there is no parental care observed in this species. As it is also reported that the selection of a suitable oviposition site is of critical importance in the reproductive success of organisms that lack parental care (Murphy, 2003b). Offspring survival should be dependent on both biotic and abiotic characteristics of the breeding site (Wells, 1977), such as food level, water depth (Crump, 1991), water temperature (Herreid and Kinney, 1967), and the presence of potential predators and competitors (Laurila and Aho, 1997; Resetarits and Wilbur, 1989). Iwai *et. al.*, (2007) reported that adult frogs are predicted to select oviposition sites of high quality where these characteristics will be appropriate for their offspring. During the present investigation, many clutches of egg even up to 27 clutches were seen in the communal egg masses adhered with each other in the same breeding site along the rivers or streams which was also observed among a common brown frog, *Rana japonica* that breeds during early spring in Japan (Iwai *et. al.*, 2007). It is suggested that communal egg masses may confer advantages because the eggs become warmer (Seale, 1982; Waldman, 1982), and the risk of predation is decreased (Håkansson and Loman, 2004). Depositing eggs in sites that have resident eggs may be advantageous for the new eggs in species that potentially form communal egg masses. Moreover, closely positioned egg masses often adhere to each other as the jelly coat absorbs water or as the water level of the pool decreases as it was observed among *Rana japonica* (Iwai *et. al.*, 2007).

During the present observation, the oviposition sites of *Koloula pulchra* were mainly temporary rock-pools or water holes which accord with the observation of Heyer (1973). This might be in order to avoid tadpoles predation, naturally the

smaller rock-pools totally lack predators of tadpoles whereas others support high densities of various predator species as in the case of common frogs in Southern Finland (Laurila and Aho, 1997). The larvae of pool anurans are unable to leave the sites selected by their parents and therefore there should be particularly strong selective pressure on adult females for the ability to discern oviposition sites in which offspring survival is expected to be high (Iwai *et. al.*, 2007). According to Heyer *et. al.* (1975) and Skelly (1997), abiotic factors (e.g. water body duration) are more important than biotic factors (e.g. competition, predation) for species' reproductive success in temporary water bodies, but predation is not independent of hydroperiod.

Microhyla berdmorei deposited their floating raft of eggs mostly in incompletely isolated lotic pools edge and slowly flowing water. Dutta *et. al.* (1990-1991) mentioned that egg masses of other Indian microhylids float on the water surface, except for *Ramanella montana* where clutches are always found adhered to a tree trunk just above the water level or they are placed on the surface of a floating leaf (Krishna *et. al.*, 2004).

It was also observed that unlike its sympatric stream breeder, *Hylarana nicobariensis* that occupies most of the main water bodies from slow to moderately fast flowing portions, the oviposition sites of *Microhyla berdmorei* mostly include the standing pools or rock-pools connected with lotic water, shaded or covered by the surrounding vegetations. This study suggested that these two species may diverge to avoid competition, reducing overlap in resource use. Crump (1971), Maiorana (1976), and Donnelly and Guyer (1994) reported that spatial or/and temporal partitioning of reproductive resources are thought to be important

mechanisms enabling syntopic taxa to avoid competition. In addition to this, it is also assumed that the selection of lotic edge pools mixed with vegetation provides better food resources, as it was also observed by Brockelman (1969). Understory vegetation in the riparian zone provides frogs with moisture, shelter and calling sites (Parris and McCarthy, 1999). Riparian vegetation also creates gradients in light levels that affect species distribution and food availability for tadpoles (Schiesari, 2004; Werner and Glennemeier, 1999). Besides, selection of vegetated microhabitats may influence predator-prey interactions and reduce predation rates on embryos as mentioned by other workers (Babbitt and Tanner, 1998; Lewis and Eby, 2002; Kopp *et. al.*, 2006). Moreover, throughout the investigation, an interesting feature observed was that *Kaloula pulchra* and *Microhyla berdmorei* selects the same microhabitat for breeding as well as oviposition at the exact location unless it was destroyed by natural calamities like floods and minor landslides. This observation suggested that the two microhylids, *Kaloula pulchra* and *Microhyla berdmorei* are very selective in microhabitat choice.

It was found that with the exception of *Kaloula pulchra*, oviposition sites were associated with the microhabitats in which the frog species were found. Calling and oviposition site selection are important determinants of reproductive success, and therefore fitness, in anurans (Lips, 1996, Resetarits and Wilbur, 1991). The isolated vernal pools adopted by *Kaloula pulchra* in this observation might be due for the survival of their offsprings from the predators like fish as reported by Hopey and Petranka (1994). Several studies of North American species suggest that vernal pond breeders have few defences against fish (Grubb, 1972; Woodward, 1983; Kats *et. al.*, 1988), whereas studies in both neotropical (Heyer *et. al.*, 1975) and temperate

(Sexton and Phillips, 1986; Semlitsch, 1988; Semlitsch and Gibbons, 1988) regions have provided data which suggest that fishes are efficient predators on temporary pond specialists.

Clutch Size: The present record on the clutch sizes of *Euphlyctis cyanophlyctis* ranged from 68 – 182 (Mean \pm SE= 136.73 \pm 4.16). Data on egg numbers per clutch are controversial; 300 – 500 (Mohanty-Hejmadi and Dutta, 1979); 150 – 500 (Mohanty-Hejmadi *et. al.*, 1983). Dutta and Mohanty-Hejmadi (1976) reported the clutch size of its sympatric species, *Rana (Hoplobatrachus) tigerina* ranged from 4660 to 6460.

The clutch sizes of *Hylarana nicobariensis* recorded ranged from 131 – 628 (Mean \pm SE= 405.62 \pm 22.75). Clutch size of other stream breeder ranids recorded ranged 600 - 2000 in *Rana longicrus* (Kam *et. al.*, 1995), 526 – 2086 in *Rana dalmatina* (Posero and Joly, 1998), and up to 1500 eggs in *Rana sylvatica* (Grant, *et.al.*, 2005).

The clutch sizes of *Kaloula pulchra* from this observation in Mizoram ranged 363 – 576, whereas Berry (1964) counted 1574 – 6330 per a single specimen in contrast with 2 – 3 dozens or even up to hundreds as reported by Leong and Chou (1999) from the same country, Singapore.

Clutch sizes in *Microhyla berdmorei* ranged between 218 and 443. Other observations on microhylids also shows wide variation e.g. Clutch size of *Microhyla ornata* ranged 500 - 575 from eastern China (Pope, 1931), 624-1207 from Iriomotejima, and 271 - 890 from Kohamajima (Matsui and Ota 1984); 61 – 1327 from Nepal (Schleich and Kästle, 2002), 220 – 910 (Shimizu and Ota, 2003); *Ramanella variegata* 574-1417 (Dutta *et. al.*, 1990-91) while that of *R. obscura* in

Sri Lanka was 557 (Morgan-Davies, 1958) and *Ramanella montana*, which has a clutch size of 108-130 (mean= 117; SD= 10.13; $n= 4$) eggs (Krishna *et. al.*, 2004).

Mohanty-Hejmadi *et. al.*, (1983) reported the number of eggs correlates with the female's nutritional state. Moreover, Ritke *et. al.* (1990) and, Morrison and Hero (2003) reported that clutch size and breeding phenology may vary over the geographic range of a wide-ranging species which may lead to variation in population dynamics.

Clutch size Vrs Female body size: No correlation was, however, observed between female body size and clutch size (where $r= 0.172$; $p= 0.189$ in *Euphlyctis cyanophlyctis*, $r = 0.230$; $p = 0.076$ in *Hylarana nicobariensis* $r=0.187$; $p=0.167$ in *Kaloula pulchra* and $r = -0.132$; $p = 0.314$ in *Microhyla berdmorei*). Therefore, the present study revealed that there was no correlation between the clutch size and female size (SVL) in all the four frog species. Although some workers reported that the clutch size and female size are correlated in some species, *Rana temporaria* (Cummins, 1986a; Joly, 1991), *Rana dalmatina* (Ponsero and Joly, 1998), *Microhyla ornata* (Matsui and Ota 1984). Other workers reported no correlation between the same e.g. *Rana (Hoplobatrachus) tigerina* (Dutta and Mohanty-Hejmadi, 1976); *Heleioporus albopunctatus* (Davies and Roberts, 2005). Dziminski (2000) reported that only three of 11 species of Australian frog species showed a positive relationship between clutch and body size.

Histology of Testis and ovary: The histological studies on the testis of all the four species during their breeding season revealed the presence of different stages of spermatocytes, spermatogonia (SG), primary spermatocytes (PS), secondary spermatocytes (SS), spermatids (ST) and spermatozoa (S). Seminiferous tubules

were more or less rounded in shape, surrounded by seminiferous epithelium which consists of spermatogonic cells and sertoli cells. Interstitial tissues were well developed and the tubules were filled with numerous slender and filamentous spermatozoa. Bundles of mature spermatozoa occur in a cluster with their tail extending into the lumen of the tubules. Bundles of spermatozoa were very prominent and attached to sertoli cells. This clearly shows that the testes are actively engaged in the process of spermatogenesis. Saidapur and Nadkarni (1975b) reported that *Rana (Euphlyctis) cyanophlyctis* exhibits a continuous type of spermatogenesis and as such the testes contain active cell nests of different stages and spermatozoa throughout the year. Sperm bundles attached to the sertoli cells and free spermatozoa in the lumen of the tubules in the present observation was also reported in *Rana (Euphlyctis) cyanophlyctis* (Yajurvedi and Hooli, 1983). Gopalakrishnan and Rajasekarasetty (1978) reported that the gametogenic activity in *Rana (Euphlyctis) hexadactyla* begins by March or April when the localities experience the pre-monsoon (southwest) showers. Sretarugsa *et. al.* (1997) and Chavadej *et. al.* (2000) identified twelve stages of germ cells, *i.e.*, primary and secondary spermatogonia, primary spermatocytes which consists of 5 stages (leptotene, zygotene, pachytene, diplotene and metaphase), secondary spermatocytes, three stages of spermatids (early, round, late) and spermatozoa were observed from the histological section of the testis of *Rana (Haplobatrachus) tigerina* and *Rana catesbeiana*. In seasonal breeding anurans, it is reported that during the non-breeding period, the number of the late stage germ cells, especially round spermatids and spermatozoa drastically decreases as well as the body and testicular weights. Some tubules are dilated and

exhibit desquamation of their epithelium; and some even show a complete breakdown (Sretarugsa *et. al.*, 1997; Chavadej *et. al.*, 2000).

The present histological studies on the ovary of all the four species during the breeding period showed the presence of growing as well as mature oocytes. Each mature oocyte was also found to be covered by a vitelline membrane and had a large amount of yolk platelets scattered in the cytoplasm. Theca externa and theca interna that cover the developing oocytes were also observed. 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. The criteria for dividing oocytes into many stages are mainly based on the size, the amount and the distribution of yolk and pigment (Kemp, 1953; Grant, 1953; Wartenberg and Gusek, 1960), and the morphology of chromosomes (Duryee, 1950). Sretarugsa *et. al.* (2001) divided the developing oocytes in adult *Rana (Haplobatrachus) tigerina* into six stages based on size, colour and histology. Ovarian cycle in all amphibians is regulated by both intrinsic and extrinsic factors. The intrinsic factors mainly include the hypophysial gonadotrophins and ovarian estrogen. The extrinsic factors are temperature, light, rainfall and relative humidity which serve as the environmental synchronizers of gametogenetic and breeding activities. Adequate supply of food and water is however, essential (Saidapur, 1989).

III. Development and Metamorphosis:

(1.) *Euphlyctis cyanophlyctis*: The result indicates that under both the natural environment as well as in the laboratory conditions, the development and metamorphosis of *Euphlyctis cyanophlyctis* completed its life cycle within 64 - 65

days where the water temperature ranged between 18.5°C – 32.5°C and 21°C – 30°C respectively, from March to August. Hatching takes place in the stage-23 after 4 days when the larva measured about 5.99 mm in length. From this observation, the hatching period and completion of life cycle was similar with the report of Jangir (2005). This skipper frog was observed to be dependent on the ephemeral standing pools or littoral regions of permanent lentic and lotic at the breeding sites to complete their life cycle. The duration of development and metamorphosis of this frog in Mizoram shows a slightly different with the observation made by Mohanty-Hejmadi and Dutta (1979), where *Rana (Euphlyctis) cyanophlyctis* approximately takes a shorter duration (46 days) in the laboratory condition and comparatively at a higher temperature which ranged between 32°C - 41°C in Orissa. However, they also reported that the duration to complete metamorphosis from fertilization varies from 15 to 60 days or even up to 94 days depending on density, nutrients and temperature. In the present investigation, it may be suggested that the difference in the rate of development between these observations might be due to geographical variation, and local ecological condition e.g. temperature, pH, relative humidity, rainfall, availability of food. Smith-Gill and Berven (1979) and, Reques and Tejedo (1995) reported that one of the most profound effects of temperature is to influence developmental growth rates and the timing of metamorphosis. Moreover, it was also assumed that the smaller size of pro-metamorphic tadpoles (average = 53 mm) observed by Mohanty-Hejmadi and Dutta (1979) than the present result (average = 64.60 mm) was due to longer duration of development and lower temperature in the present observation. The average size of newly metamorphosed froglet in the present study was about 20 mm which was slightly larger than 17 mm froglet observed by

Mohanty-Hejmadi and Dutta (1979). Korbeck Jr. and McRobert (2005) mentioned that increases in temperature result in faster rates of larval development and growth, a reduction in the time it takes to reach metamorphosis, and a smaller body size at metamorphosis. Exposure of anurans (frogs and toads) to higher temperatures generally increases growth rates and decreases maturation times, therefore affecting reproductive output (Hadfield, 1966; Lillywhite, 1970; Smith, 1976).

(2.) *Hylarana nicobariensis* : Literature surveys revealed that there is no other work on the development and metamorphosis of *Hylarana nicobariensis* except for some descriptions on adult and larvae (Boulenger, 1920; Chanda, 2002; Ming, 2005) and the present result indicated that the development and metamorphosis of *Hylarana nicobariensis* takes place during the cold winter season (October to February) in Mizoram and completed its life cycle within 74 - 75 days, both in the natural environment and in the laboratory conditions where water temperature ranged between 12°C – 24.5°C and 13°C – 25°C, respectively. Hatching of the larva takes place in the stage-21 after 6 days. It was observed that this stream breeder depends on the lotic and the adjacent water bodies for their breeding sites and to complete their life cycle. Observation on the embryonic development and hatching duration of *Hylarana nicobariensis* was found to accord with another winter breeder, *Rana longicrus* from Taiwan (Yuan, 1950; Kam *et. al.*, 1995) that metamorphosed into adults within 50 – 60 days under water temperature 19°C – 20°C, and a stream breeder, *Rana japonica* from Japan (Iwai *et. al.*, 2007) that require three days to develop from newly-deposited eggs to Gosner's stages 15 – 17, five days to hatching, and 8 – 9 days to begin feeding. Dutta and Mohanty-Hejmadi (1976) reported that the Indian bull frog *Rana (Hoplobatrachus) tigerina* takes 33 days to

complete life history where hatching takes place in about 24 hrs, and well developed hindlimbs (maximum length=38.8mm) in the laboratory condition with temperature 28°C - 36 °C. The Indian burrowing frog *Rana breviceps* takes 45 days to complete life history where hatching takes place in about 44 hrs, and well developed hind limbs (maximum length) attained at stages 38 - 40 (38.8 mm) in the laboratory condition with temperature 30°C - 33°C (Mohanty-Hejmadi *et. al.*, 1979a). The slower rate of development with larger tadpoles (40.89 mm) at stage 40 in *Hylarana nicobariensis* than other Indian ranids might be due to the breeding season that coincides with cold climatic condition where the water temperature ranged between 12°C – 24.5°C. Moreover, laboratory studies 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). Dodd and Dodd (1976), Smith-Gill and Berven (1979) and Merilä *et. al.*, (2000) also reported that the effect of temperature on development is as expected from laboratory studies; a higher rate at high temperatures. At present, however, there is little information on the development and metamorphosis of stream breeders as compared to pond breeders. For instance, tadpoles of *Hylarana nicobariensis* (unlike *Euphlyctis cyanophlyctis*) are found mostly in large bodies of water and as such do not face the levels of desiccation and crowding. Therefore, tadpoles of *Hylarana nicobariensis* take much longer (2½ months) to metamorphose, whereas *Euphlyctis cyanophlyctis* metamorphosed within 2 months approximately. This observation agreed with the comparative studied on the duration for metamorphosis made between *Rana temporalis* (3-4 months) and *Tomopterna breviceps* one month or so) by Gramapurohit *et. al.* (2004).

(3.) *Kaloula pulchra* : By conducting studies on the embryonic development and metamorphosis of *Kaloula pulchra* both in the natural and laboratory conditions with the water temperature ranged between 14.5°C – 29°C and 16°C – 28°C respectively, it was found that the completion of life cycle occurred within 47 days. Hatching of the larva was observed in the stage-20 after 29:30 hrs when the larva measured about 4.33 mm in length. The dependent of life cycle in the temporary rock-pools and ephemeral water bodies is in agreement with Heyer (1973), and Leong and Chou (1999). The hatching period and duration of life cycle was very close to other Indian microhylids like, *Uperodon systoma* that hatched in about 30 hr after fertilization and completed the life history within 51 days in the laboratory condition (Mohanty-Hejmadi *et. al.*, 1979), *Microhyla ornata* that also hatched in 34 hrs and accomplished its life cycle within 49 days (Mohanty-Hejmadi *et. al.*, 1980) and *Ramanella variegata* hatched in 28 – 30 hrs with 32 days life cycle duration (Dutta *et. al.*, 1990-91). The timing of metamorphosis in this report (47 days) shows comparatively a bit longer period than 4 weeks as mentioned by Leong and Chou (1999) in the temperate region of Singapore. Average diameter of the eggs (1.48 mm) in our observation was slightly smaller than their reports (1.5 mm - 2 mm). Most of the larval characteristics are in agreement with their observation except for the size of larger tadpoles in the present study which can be explained by the fact that low temperatures retard differentiation more than growth, thereby increasing stage-specific size (Smith-Gill and Berven, 1979). As a result, larval anurans grown at cold temperatures have prolonged developmental periods but they are also larger as metamorphs than conspecifics grown at warmer temperatures. This phenomenon makes up one of the most general rules for ectotherms (Atkinson, 1994 and 1996).

However, in comparison with the other three species (*Euphlyctis cyanophlyctis*, *Hylarana nicobariensis* and *Microhyla berdmorei*), the life cycle of *Kaloula pulchra* is comparatively shorter and it might be referred to as ‘explosive breeder’ used by Ritcher and Seigel (2002) for those species that breed in temporary water bodies where metamorphosis and development is completed within a short hydroperiod. The observed rapid developmental rates might be advantageous for these microhylid species in ephemeral habitats, which allow larvae to metamorphose quickly and escape desiccation and reduce exposure to aquatic predators and diseases as reported also in other frog species (Low, 1976; Newman, 1992; Denver, 1997, 1998). Tadpoles from small temporary ponds have been reported to spend more time feeding and to develop faster than tadpoles from large permanent ponds, where the larvae spend more time hiding from predators, and, as a consequence develop more slowly (Peltzer and Lagmanovich, 2004).

(4.) *Microhyla berdmorei*: As Chanda (2002) reported that they are very rare species and no data are available on their natural history and more extensive field study is necessary to know the actual status of the species, except for some descriptions on adult and larvae (Smith, 1924; Parker, 1934; Bourett, 1942; Inger, 1966; Leong, 2004; Inthara *et. al.*, 2005). The present findings indicated that the embryonic development and metamorphosis of *Microhyla berdmorei* takes place during the cold winter season (October to March) in Mizoram and completed its life cycle within 109 days, both in the natural environment and in the laboratory conditions where water temperature ranged between 12°C – 24.5°C and 13°C – 24°C, respectively. Krishna *et. al.* (2004) observed *Ramanella montana* to complete its life cycle within 160 days where hatching was documented after 8 days under 21.5°C - 22°C. In this

investigation, the longer duration of life cycle in *Microhyla berdmorei* might be due to the lower water temperature similar to *Ramanella montana* (Krishna *et. al.* 2004). It was observed that *Microhyla berdmorei* depends on the shaded standing portion of lotic and the adjacent water bodies for their breeding and to complete their life cycle in order to avoid water current, so that the embryos would not be washed away. It was also suggested that this microhabitat might be to protect the developing embryos from direct sunlight and higher temperature. Hatching of the larva takes place in the heart beat stage (*i.e.* stage-19) after 1 day and 3 hrs as it was observed on *Microhyla ornata* by Shimizu and Ota (2003) after 1 day and 3 hrs. Literature survey shows that, there is a large variation in the time taken for the completion of life cycle among the microhylids. Mohanty-Hejmadi *et. al.* (1979b) reported the larvae of *Uperodon systoma* hatched in about 30 hrs after fertilization and completed the life history in 51 days under the laboratory condition with temperature 30°C - 33°C. Hatching of *Microhyla ornata* within 38:30 hrs and metamorphosis within 49 days under 29°C – 30°C was observed by Mohanty-Hejmadi *et. al.* (1980), but takes 40 days to complete metamorphosis under 25±1°C (Shimizu and Ota, 2003). Dutta *et. al.*, (1990-91) reported the durations of hatching and life cycle in *Ramanella variegata* as 28 hrs to 30 hrs and 32 days respectively. Sanuy *et. al.*, (2008) mentioned that higher temperature treatments determined both higher developmental rates and higher growth rates. The maximum size (24.88 mm) of larva was observed in the 40 stage which is in agreement with other workers (Mohanty-Hejmadi *et. al.*, 1979b, 1980; Dutta *et. al.*, 1990-91; Shimizu and Ota, 2003).

From the above observations on the embryonic development and metamorphosis of these species, it was confirmed that the ranid (*Euphlyctis*

cyanophlyctis) and the microhylid (*Kaloula pulchra*) take place from the onset of monsoon i.e. late February or March to the mid-monsoon (August) but a shorter period in the case of *Kaloula pulchra*. The two stream breeders, the ranid (*Hylarana nicobariensis*) and the microhylid (*Microhyla berdmorei*) were observed to undergo development and metamorphosis during cold winter months i.e. October to February and October to March, respectively.

From the present investigation, it was found that the development of *Euphlyctis cyanophlyctis* showed variation in both temperature and pH of water as reported by Boulenger (1920), that the tadpoles of *Euphlyctis cyanophlyctis* showed plasticity and vary considerably with their environment. The water temperature for the development and metamorphosis of *Euphlyctis cyanophlyctis* in the natural and laboratory ranged from 18°C – 32.5°C, of *Kaloula pulchra* from 14.5°C – 29°C, *Hylarana nicobariensis* from 12°C – 25°C and *Microhyla berdmorei* from 12°C – 24.5°C that might be the ambient temperature for each species. For anuran tadpoles, temperature can be a critical environmental factor, influencing activity patterns, physiology, growth, and development (Duellman and Trueb, 1994; Smith-Gill and Berven, 1979; Ultsch *et. al.*, 1999). pH of water for the development of *Euphlyctis cyanophlyctis* in the natural and laboratory ranged from 6.4 – 7.6, of *Kaloula pulchra* from 6.0 – 6.7, *Hylarana nicobariensis* from 6.3 – 7.4 and *Microhyla berdmorei* from 6.3 – 7.4 which might be the optimal pH range for each species. From this study, it is believed that extreme value of pH may be deleterious for the developing aquatic embryo as mentioned by Freda and Dunson (1985a) that, low pH can have important physiological as well as ecological consequences for amphibian populations. Pough and Wilson (1977) also reported that amphibian embryos are

more sensitive to low pH at higher temperatures. 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). 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. Although some workers have suggested that some populations of certain species of frogs show a greater tolerance to low pH (Gosner and Black, 1957; Clark and Lazerte, 1987), this is not a general phenomenon (Clark, 1986). Very little is known about the potential of amphibians to adapt to low pH conditions (Andren *et. al.*, 1989). Several experimental studies revealed negative effects of acid water on embryos and larvae of different amphibian species (Andren *et. al.*, 1988; Rowe *et. al.*, 1992) and also reduced the survival probability as well as increased the frequency of developmental anomalies in some amphibian species (Pahkala *et. al.*, 2001). 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 embryo and high embryonic mortality. Glos *et. al.* (2003) reported that acidification of habitat was thought to have a major impact on amphibians and the structure of their populations, and lethal pH values for different amphibian species in the developmental stage range between pH 3.5 and pH 5. 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). However, 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. Low pH has been shown to decrease growth and developmental rates or to delay metamorphosis in other ranids like *Rana temporaria* (Cummins, 1986b, 1989; Beattie and Tyler-Jones, 1992), and *R. sylvatica* (Sadinski and Dunson, 1992; Rowe *et. al.*, 1992; Horne and Dunson, 1995). The pH value of microhabitat choice never goes very low or high as some species seem to avoid naturally acid habitats (e.g., Freda and Dunson, 1986). In general, the negative effects of acid waters on amphibians are considered an important factor for the decline of amphibian populations in the northern hemisphere (Blaustein and Wake, 1990). Differences in acid tolerance are known to exist between species, between populations of one species, and between individuals of one population. Interspecifically, species of naturally acid habitats (e.g., bogs, blackwaters) tend to be more acid tolerant than sympatric species of neutral habitats (Picker *et. al.*, 1993). Some species seem to avoid naturally acid habitats (e.g., Freda and Dunson, 1986). Intraspecific differences were shown for *Rana sylvatica* (Gosner and Black, 1957) and *Ambystoma maculatum* (Clark and Lazerte, 1987). In these species, populations of naturally acid habitats were more acid tolerant than populations of neutral habitats. However, this is not a general phenomenon (e.g., Clark, 1986).

Hatching stages for the embryo of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* were stages 23, 21, 20 and 19, respectively. After hatching, each tadpole undergoes a period of growth and development prior to metamorphosis into the terrestrial life stage as mentioned by Rowe and Freda (2000). The duration for the completion of life cycle in *Euphlyctis cyanophlyctis* was 64 - 65 days, *Kaloula pulchra* 47 days, *Hylarana nicobariensis* 74

- 75 days and *Microhyla berdmorei* 109 days. This shows that difference in the duration of embryonic and larval period may also be depending on species as reported by Rowe *et. al.* (2001).

IV. Food and feeding in relation to oral structures and intestines:

Based on the methods utilised by tadpoles for trapping food, several ecological or adaptive types have been recognized by Orton (1953), a scheme subsequently revised by Duellman and Trueb (1994). The major types of concern in the study species are 1: Obligate suspension-feeders (Orton's type 2), nektonic filter-feeders to which virtually all microhylids belong, including *Kaloula pulchra* and *Microhyla berdmorei* and 2: facultative suspension-feeders (Orton's type 4), the generalised pond (nektonic) type, represent by the larvae of both *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*.

Larval foods: Observation on the present results showed that tadpoles of all the four species started feeding from stage 25 onwards. During the early stages of feeding, they feed mostly on detritus and plant materials and during the later stages of feeding they consumed both phytoplankton and zooplankton. Tadpoles soon stop feeding at stage 42 and after metamorphosis the froglet start feeding on a carnivorous diet.

Euphlyctis cyanophlyctis: Observations on a few quantities of blue-green algae with numerous chlorophyceae and bacillariophyceae in the tadpole intestines of *Euphlyctis cyanophlyctis* was in agreement with Sinha *et. al.* (2000). The detritus packed along the length of intestine was an indicative of its habitat as a benthic detritus feeder which was also observed by Das (1994) and Khan (2000). Nibbling at vegetation and carcasses of other tadpoles which was also an indicative of

larvivorous as described by Khan (2000) was observed during this study period. No cannibalistic behaviour was observed among them, and the cannibalism reported in McCann (1932) and then in Bourret (1942) is obviously to have been based on observation of tadpoles of *Hoplobatrachus tigerinus* (Grosjean *et. al.*, 2004). Wide spectrum of food choices indicated that they inhabit various types of habitat, stream, ponds, lake, lotic-connected shallow standing pools and ponds.

Hylarana nicobariensis: Filamentous Chlorophyceae (*Cladophora*, *Ulothrix*, *Mougeotia*, *Oedogonium*, *Sirogonium*, and *Spirogyra*) were predominant in the intestine of tadpoles collected from swift waters, in addition to these, non-filamentous Chlorophyceae (*Closterium*, *Cosmarium*, *Docidium*, *Staurastrum*, *Stauroneis*, and *Pediastrum*) along with other items were recovered from those tadpoles of stream-connected standing pools. During this study period, since the blooming of *Cladophora* coincides with the larval development of *Hylarana nicobariensis*, it was observed that the tadpoles were frequently feeding on these filamentous green algae that probably served as an excellent nutrient. Pryor (2003) reported that even a cleaned *Cladophora* without the supplemental sources of nutrients such as phytoplankton and zooplankton, detritus, and prokaryotes are sufficient for the growth and development of tadpoles.

Kaloula pulchra: As they were mainly collected from small rock-pools within a short period, Bacillariophyceae and Cyanophyceae (especially *Anabaena*) are comparatively more abundant than other food items. The preference of Bacillariophyceae and Cyanophyceae in the food habits of this species may be that the only available food items in their microhabitat that enhanced the faster

developmental rate within a short duration. From the observation of Pryor (2003), it was evident that diatoms alone are sufficient for the growth and development of tadpoles and diatoms contain more calories (in the form of fat and protein) than other algae and hence they are a preferred food (Kupferberg 1997b). Moreover, it was observed that the filamentous blue-green alga *Anabaena* promoted better growth in Bullfrog tadpoles (*Rana catesbeiana*) than the other algal species (Pryor, 2003).

***Microhyla berdmorei*:** The larval food mainly consists of Bacillariophyceae followed by Chlorophyceae, Cryptophyceae, zooplankton, Euglenophyceae, detritus and Cyanophyceae. Among the food items, Chlorophyceae, *Cladophora* and *Mougeotia* are predominant. In contrast to its sympatric *Hylarana nicobariensis*, the tadpoles of *Microhyla berdmorei* were observed to feed more frequently on Bacillariophyceae as mentioned by Khan and Mufti (1994) that every species of sympatric tadpoles differ not only in its oral disc morphology, but also in utilization of a particular part of the common food base available in pond ecosystem.

From the current examination on the food habits of these four anuran larvae, it is evident that despite common assumptions of the reliance of anuran larva on algal diets per se, components of aquatic ecosystems other than photosynthetic algae (e.g. detritus, diatoms, bacteria, protozoa) may represent valuable sources of food to tadpoles as reported by other workers (Krogh, 1931; Burke, 1933; Li and Lin, 1935; Savage, 1952; Wassersug, 1975; Ahlgren and Bowen, 1991; Kupferberg *et. al.*, 1994; Kupferberg, 1997b; Thibaudeau and Altig, 1999; Pryor, 2003). Most tadpoles have been regarded primarily as herbivores (Kupferberg *et. al.*, 1994), whereas some species are known to grow more efficiently with animal materials (Alford, 1999).

Moreover, Hoff *et. al.*(1999) reported that anuran larvae are omnivores consuming a variety of food; detritus, viruses, bacteria, protists, algae, pollen, fungi, small animals, and so on. The relative suitability of food items should vary according to species (Iwai and Kagaya, 2005a).

Although the tadpoles of *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis* have been characterized as plankton feeders, they can also graze on substrate with their keratinized jaw sheaths and teeth, as evidenced by the food items in the digestive tract. Indeed, 40% of tadpoles fed only detritus survived to metamorphosis (Kupferberg *et. al.*, 1994). In these four species, the range of food particle size, as well as the qualitative composition of the diet in tadpoles, is highly variable, and is linked to food availability. Therefore, the present investigation showed availability and composition of food directly affects performance of anuran larvae as reported by Beck (1997), Brown and Rosati (1997), and Kupferberg (1997b). However, Heyer (1973) and Inger (1986) mentioned interspecific variation in the sizes of food particles ingested. Although most anuran larvae are thought to feed indiscriminately, some studies postulate a possible resource selection, which would permit a differentiation in the trophic niche (Steinwascher and Travis, 1983; Taylor *et. al.*, 1995). In addition to this, the diet of tadpoles in natural habitat can be influenced by food availability and presence of their competitors and predators (Bridges, 2002; Relyea, 2004; Skelly and Golon, 2003). Although, in some instances diurnal/nocturnal habits may have contributed to niche differentiation, it did not happen in all cases, as most tadpole pairs with high superposition in microhabitat use are all active during the day. Pharyngeal and buccal structures determine the size of food items on which tadpole species feed most efficiently, so that partitioning of

food particles by size is possible among tadpoles. Morphological and physiological adaptations of tadpoles can also determine food acquisition, as filtering or scratching, as well as microhabitats where feeding activities take place (Duellman and Trueb 1994). Notwithstanding, tadpoles are better thought of as opportunistic omnivores or detritivores than as specialized feeders (Hoff *et. al.* 1999), which makes niche differentiation through food partitioning unlikely. Indeed, Heyer (1973, 1974) found space to be much more important than food in niche partitioning by tadpoles in ponds from Thailand. Since the food condition will change according to habitat, larval food habit of a frog species should relate to its distributional pattern; a species might have evolved to be more successful with the food condition of their suitable habitat, or a species may choose the habitat where their suitable food is abundant (Iwai and Kagaya, 2005b).

Oral apparatus: In the case of *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*, development of mouthparts began soon after hatching and at stage 25 there is appearance of minute horny teeth and the tadpole start feeding.

Euphlyctis cyanophlyctis: The described observations showed that the LTRF (labial teeth row formula) in *Euphlyctis cyanophlyctis* was 1/2 which was also reported by other workers (Mohanty-Hejmadi and Dutta, 1979; Khan, 1982; Khan and Mufti, 1994; Khan, 2000; Saidapur, 2001; Grosjean *et. al.*, 2004), but shows a minor difference with 1/2(1) as described by Das (1994). The explanation for this dissimilarity is that structural differences are to be expected among species that utilise different food resource, because labial teeth are subjected to break up particles from algal mats and periphyton and/or aquatic macrophytes to create suspensions

(Duellman and Trueb, 1994). The teeth formula was consistent throughout the development up to stage 41, unlike other anurans where the number of teeth row shows dissimilarity during growth and development. Das (1994) reported that the thick and heavy beaks (jaw sheaths) are thought to be adaptations for scraping algae from rocks. Suggestion on shape, pointed (non-cusped) might be for scraping, piercing and tearing, the large jaw with its strong medial upper projection act or help/screen/filter/ select types of food during the feeding process. The absence of papillae in the middle portions of the upper and lower labia might serve as the entrance for the food. The presence of dorsal and ventral gaps is the pattern also seen among most bufonids and a few hylids, mantellines, ranids and rhacophorids (Altig and McDiarmid, 1999). Serration on the jaw sheaths triangular and pointed may be for cutting larger food particles.

Hylarana nicobariensis: In the present observation, the LTRF of *Hylarana nicobariensis* was 2(1)/3(1) which shows differences from LTRF 1/3(1) reported by Schijfsma (1932) and 1/2(1), reported by Ming (2005). Variation in mouthpart morphology may be due to geographic location e.g. *Rana alticola* from the northeastern India (Annandale, 1912; Sahu and Khare, 1983), peninsular Thailand (Smith, 1924), and Phang Nga Province, Thailand (Grosjean *et. al.*, 2003), microhabitat as in *Rana curtipes* (James *et. al.*, 2000), or inter-population variation as in the common frog *Rana temporaria* (Vences *et. al.*, 1998, 2002). However, Vences *et. al.*, (1998) stated that differences in the number labial keratodont rows and lingual papillae have been considered as indications of taxonomic distinctness of the respective populations. The present observation shows that the presence of

ventral gap in the marginal papillae which is taxonomically and ecologically common pattern as mentioned by Altig and McDiarmid (1999). Those gaps on the A-2 and P-1 may allow larger excursions of the jaw sheaths during feeding; adjacent tooth rows that have only narrow or no gaps perhaps signal smaller jaw excursions during feeding.

***Kaloula pulchra* and *Microhyla berdmorei*:** This present finding on the absent of jaw sheath and keratodonts in the tadpoles of *Kaloula pulchra* and *Microhyla berdmorei* agrees with a report made by Inthara *et. al.* (2005), where *Microhyla ornata*, *Microhyla butleri*, *Microhyla pulchra*, *Micryletta inornata*, and *Glyphoglossus molossus* are also included. Rao (1933) also reported the total absence of the teeth in the tadpoles of *Kaloula* and *Microhyla*. Das and Coe (1994) also reported the absent of denticles in *Microhyla rubra*, *Microhyla ornata* and *Uperodon systoma*. In the present observation, the umbelliform disc tadpole of *Microhyla berdmorei* has a large, infolded semicircular structure at each corner of the mouth that appears derived from the lower labium as it is also seen in the tadpoles of *Microhyla heymonsi* (Altig and McDiarmid, 1999) and *Microhyla ornata* (Khan and Mufti, 1994). Altig and McDiarmid (1999) reported that an umbelliform or upturned oral disc appears as convergent trait in tadpoles of some arthroleptids, dendrobatids, hylids, mantelline rhacophorids, megophryids, and microhyline microhylids. Most umbelliform tadpoles occur in the backwaters of lotic systems. Tooth rows and jaw sheaths are reduced to absent in these forms, and large ridgelike papillae that project radially from the mouth are common.

As it is seen in both *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*, each keratinized labial tooth is derived from cells in the base of the tooth ridge and consists of three indistinct regions: a distal head, an intermediate body, and a basal, hollow sheath (Gosner, 1959). A single tooth row per ridge (= uniserial) is seen in both *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis* throughout their larval development. Altig and McDiarmid (1999) mentioned that most tadpoles have a single tooth row per ridge (= uniserial), but tadpoles with bi- (e.g., discoglossids) and even tri- or multiserial rows are known. Tooth densities of 30-100/mm, with higher counts in lotic tadpoles, are typical, and the number of cusps range from 0 (simple spike) to at least 18. Biserial sections in typically uniserial rows are not uncommon. The jaw sheaths of both the larvae of *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis* are of a typical tadpole (Altig and McDiarmid, 1999), with broad-based and short serrated edge. Jaw sheaths are formed by the fusion of palisades of keratinized cells along their lateral margins (Kaung, 1975; Kaung and Kollros, 1976). Jaw sheaths with 30-80 serrations/mm are typical, and higher counts are known in lotic forms. In few cases (e.g., some *Amolops* and *Boophis*), serrations on the edges are only 5-8/mm (Altig and McDiarmid, 1999). Likewise, only a few workers (e.g., Altig and Johnston, 1989) have attempted to place the sizes, shapes, and serration patterns of jaw sheaths into a functional context. Even though the upper and lower jaw sheaths in a typical tadpole are similar in size and probably serve as gouging/biting/scraping structures, their striking differences in shape and the extensive array of morphologies across taxa suggest an innumerable variety of performance abilities. Altig and Mc Diarmid (1999) reported that the bases of submarginal papillae usually are circular which supports the present observation on

the two species, *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*. The functional roles that have been proposed for oral papillae fall into two basic categories: chemosensory and tactile receptors and structures that control water flow (Van Dijk, 1981), enhance attachment to substrates (Altig and Brodie, 1972; Gradwell, 1971, 1975b), modify the shape of the oral disc during feeding, and manipulate food and substrate particles. A great number of papillae were associated with an efficient filtration capability at the level of buccal cavity, whereas their absence presupposes either the lack of this capability or the presence of compensatory structures, such as dense gill filters (Wassersug, 1980). The infralabial papillae would act as respiratory or sensory structures, or they might also have mechanical interactions with food and water currents. They move in concert with the jaws, erecting when the mouth is at maximum gape, and flicking caudally as the rostrodonts begin to close (Wassersug and Yamashita, 2001).

In the present study the oral structure of the four species was found to be different from each other. The number of teeth rows in both *Hylarana nicobariensis* i.e. 2(1)/3(1) and *Euphlyctis cyanophlyctis* i.e. 1/2 was also found to be different. Altig (2007) reported that a labial tooth row formula (LTRF) of 2/3 is the most common configuration, but the number varies from 0/0 - 17/21 in many combinations throughout Anura. Variation on oral morphology in the four species agrees with the study that the structure of the mouth and buccal cavities of anuran larvae are highly adaptive and correlated with feeding ecology (Wassersug, 1976). Khan and Mufti (1994) reported that oral morphology of anuran tadpoles differ specifically reflecting adaptive radiations of each species to exploit different parts of the available food base in the pond ecosystem. The present findings reported that the

LTRF in both species is consistent up to metamorphic stages. Altig and McDiarmid (1999) reported that some tadpoles add rows proximally to the rows of the 2/3 formula on the anterior labium and distally on the lower labium. Others add rows distally on both labia. Position of oral disc on the body of tadpoles reflects its feeding ecology, food preferences and methodology employed by it for food acquisition (Altig and Johnston, 1989; Khan and Malik, 1987a; Khan, 1991). Wassersug and Yamashita (2001) found that keratodonts help to hold the oral disc and rostroodonts against the substrate and they are released from it in a definite order. Some suggested functions include acting as a broom, serving as a current generator, breaking up mucilaginous layers, sieving particles, combing strands into alignment for easier cutting (Altig and Johnston, 1989), piercing plant cells, rasping food particles from a substrate (Savage, 1952), holding food (Luckenbill, 1965), attaching to a substrate (Altig and Brodie, 1972; Gradwell, 1975a&b), and trapping food (Tyler, 1963). The absence of keratodonts excludes surface-rasping in some species and, conversely, the presence of robust rostroodonts and multiple tooth rows signals food removal by surface rasping (Candioti, 2004). During the early stages of feeding, they feed mostly on detritus and plant materials and during the later stages of feeding they consumed both phytoplankton and zooplankton. Tadpoles soon stop feeding at stage 42 and after metamorphosis the frog-let start feeding on a carnivorous diet. From this observation, it is hypothesized that 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).

Adult Foods:

***Euphlyctis cyanophlyctis*:** The findings on large amount of coleopterans and other insects from the stomach of adults *Euphlyctis cyanophlyctis* was strongly supported by Mohanty-Hejmadi and Acharya (1979), and Das and Coe (1994). Since the adults having recurved teeth are found to prey on hard-prey (e.g. coleopterans), *Euphlyctis cyanophlyctis* are referred to as ‘durophagus’ (hard-prey eaters) or consume large prey (Das and Coe, 1994). The occurrences of both aquatic and terrestrial insects, and plant materials in our specimens also agreed with Mohanty-Hejmadi and Acharya (1979). Earlier, Mohanty-Hejmadi *et. al.* (1979c) have reported that this frog being both diurnal and nocturnal feeds both during day and night but intake is higher at night.

***Hylarana nicobariensis*:** High percentage of terrestrial food items in *Hylarana nicobariensis* agree with the findings of an earlier study on stream breeding ranids, *Rana longicrus* (Kam *et. al.*, 1995) and *R. swinhoana* (Kam *et. al.*, 1998). Similarly, it has been reported by Pope and Matthews (2002) that adults of *Rana muscosa* prey on a variety of organisms, including aquatic and terrestrial invertebrates and anuran larvae. Erfemeijer and Boeadi, (1991) reported that *Rana chalconota* feeds mainly on Hymenoptera (especially Formicidae) and other terrestrial as well as aquatic insects. Coleopterans, Dipterans and Hymenopterans are also known to be the major foods of *Rana arvalis* and *R. dalmatina* (Aszalós *et. al.*, 2005). This analysis shows that terrestrial insects were the most abundant items in their diets. This indicates that even though they are regarded as riparian frogs, they must have foraged in terrestrial habitats especially on the banks of the stream which are heavily sheltered by bushes.

Kaloula pulchra: The stomach content suggested that, insects belonging to Isoptera, especially to family Termitidae (non winged forms) and winged termites were the most important food of these frogs. The highly abundance of termites in their food items would indicate that it might be either selective for termites or their emergence coincides with the emergence of termites following rains. Emerson (1976) reported that burrowing frog populations usually inhabit concentrated food areas. The concentration on termites and lack of mud supports the view that *Kaloula pulchra* mainly feeds at the surface rather than underground as observed in *Uperodon systema* (Mohanty-Hejmadi and Acharya, 1979).

Microhyla berdmorei: Small insects belonging to Hymenopterans, especially to family Formicidae, e.g. ants comprised majority of the food items as it was observed in other microhylids like *Microhyla ornata* (Mohanty-Hejmadi and Acharya, 1979; Das and Coe, 1994), *Microhyla heymonsi* (Erfemeijer and Boedi, 1991) and *Uperodon systema* (Das and Coe, 1994). Other items, isopteran are also reported from the diet of *Microhyla ornata* and *Uperodon systema*, coleopteran from *Microhyla rubra*, *Microhyla ornata* and *Uperodon systema* (Das and Coe, 1994).

The findings on the food composition of the four species consisted terrestrial and aquatic insects. Terrestrial and aquatic insects have been reported as preferential anuran prey items in several studies conducted over the past 20 years (Toft, 1980, 1981; Van Sluys and Rocha, 1998; Anderson *et. al.*, 1999; Cogalniceanu *et. al.*, 2001). From the insect orders, Santos *et. al.*, (2004) identified Odonata, Coleoptera, and Hymenoptera eaten as prey items by almost all the eight species of anurans from rainforest fragment in northeastern Brazil. However, there was minor difference on

the food choice among the four species, *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei*. Sympatric species may be subjected to a similar spectrum of potential prey, but not necessarily feed on the same items, due to differences in taxonomy, patterns of microhabitat use, or body size (Van Sluys and Rocha, 1998). Both in aquatic and terrestrial habitats, different patterns of habitat partitioning can occur, in which species may have either distinct or shared preferences. Predation may also play a role in determining prey species distribution (Wisheu 1998), a phenomenon often observed within aquatic habitats (Baker and Ball, 1995; Brown 1991; Power, 1984). If the available niche space is occupied by a species set, species are expected to share the available resources by either having different preferences (Streams, 1987), contracting their niches (Southerland, 1986) or tolerating niche overlaps with coexisting species (Pianka, 1973).

The occurrence of leaves and other debris in the stomach of all the four species might have been ingested incidentally with the prey as also observed by Mathew and Andrews (2001). The present results on the comparatively smaller size foods among adult microhylids might be reflected by their head shape, which is important in the number and size of prey they consumed. Other studies (Inger and Marx, 1961; Toft, 1982) have found a significant body size-prey size correlation between but not within, species. This lack of correlation is probably the result of the small range of body sizes within species relative to among species (Parmalee, 1999). This supported the present study on the size of these two microhylids, where *Microhyla berdmorei* is the smallest among the four species with SVL ranged from 28.55 mm - 35.7 mm, whereas *Kaloula pulchra* (SVL = 57.74 mm - 69.94 mm) is

the largest among the four species. Parmalee (1999) reported that regardless of overall size, anurans with narrower heads and shorter jaws eat more, and smaller prey items. A wide head and longer jaw contributes to a larger gape (Emerson, 1985), which is necessary to consume relatively large prey. A shorter jaw may facilitate a faster feeding cycle and may be advantageous for an animal that needs to consume large quantities of relatively low quality prey such as ants. Clearly diet is a complex phenomenon that is affected by body size and head shape, phylogeny, microhabitat, and foraging behaviour (Parmalee, 1999). Present observation on the foods of *Kaloula pulchra* and *Microhyla berdmorei* shows that, although microhylids are ant specialist, ants and termites are not the only prey they consume, but also small beetles, dipterans, small orthopterans and so on as reported by Parmalee (1999). Several other studies have also demonstrated ontogenetic changes in diet (Christian, 1982; Donnelly, 1991; Flowers and Graves, 1995; Labanick, 1976; Lima, 1998).

Histological study on the intestines of tadpole and adult:

The present investigation showed that the tadpole intestine of all the four species consists of two spiral coils: the outer coil (duodenum and anterior ileum) reverses direction at the switchback point followed by the inner coil (posterior ileum and colon), which terminates at the rectum. Shortening of the intestine during spontaneous metamorphosis is observed in all the four species (by 85%, 83%, 80% and 82% in *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei*, respectively) which is supported by the shortening of premetamorphic gut length to its one third by completion of metamorphosis and the

shortening occurs uniformly along the intestine's length (Pretty *et. al.*, 1995). The abrupt shortening of the anuran gut during metamorphosis has been well documented. Schreiber (2005) reported that in 1 week, at the climax of metamorphosis, the intestine shortens 58–90% depending on the anuran species, 42.3% for *Phrynohyas resinifictrix* (Wilczyńska *et. al.*, 2004), 58.15% in *Rana temporaria*, 75% in the *Xenopus laevis* (Schreiber, 2005), 82.2% in *Rana catesbeiana* (Janes, 1934), 84% in *Rana catesbeiana* (Carver and Frieden, 1977) and to as high as 90% in *Alytes obstetricians* (Dauca and Hourdry, 1985). In the present study, shortening of the intestine during spontaneous metamorphosis accompanied by a change in cross-sectional morphology from a single layer of cuboidal epithelial cells into a complicated layers consisting of secondary epithelium, intestinal fold lined with numerous microvilli agreed with other anurans studied so far (Schreiber, 2005). Development of intestinal folds increased the absorptive surface of the small intestine (Wilczyńska *et. al.*, 2004). As anurans transform from an omnivorous tadpole to a carnivorous frog by metamorphosing, the long small intestine, which consists of predominantly a single tubular layer of larval epithelium with very little connective tissue or muscle, reduces in its length by about 90% (Marshall and Dixon, 1978) and is replaced with the adult type of complex organ comprised of a multiply folded epithelium with elaborate connective tissue and muscle, accompanied by the programmed cell death of larval epithelium (Bonneville, 1963; McAvoy and Dixon, 1977). Both apoptotic bodies derived from larval epithelial cells and intraepithelial macrophage-like cells suddenly increase in number around the beginning of spontaneous metamorphic climax (Nakajima, *et. al.*, 2005). These structural differences between larval and adult intestines presumably reflect changes

in the physiological functions between herbivorous tadpoles and carnivorous frogs (Shi, 2000). The structural changes of musculature are formed in rearrangements of smooth muscle cells, but no proliferation of these cells with the metamorphosis (Akiyoshi *et. al.*, 2002). In changes which take place in the alimentary canal during larval development of anurans are under control of many extrinsic (ecological features of an environment) and intrinsic (thyroxine activity) factors (Wilczyńska *et. al.*, 2004). Therefore, it is found that during metamorphosis of the four species, *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* there are both microscopic changes in the intestines at the histological level and macroscopic changes in its overall length as also reported by Pretty *et. al.* (1995).

CONCLUSION

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The present study synthesizes information about the distribution, breeding behavior and development of ranids (*Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*) and microhylids (*Kaloula pulchra* and *Microhyla berdmorei*) prevalent in Mizoram. It was found that all the four species had a wide range of distributions and can adapt to low and high altitude. During this study, different parts of Mizoram were surveyed and collections were made from all the eight (8) districts (i.e. Aizawl, Kolasib, Champhai, Mamit, Serchhip, Lunglei, Lawngtlai and Saiha) of Mizoram. From the northern part of Mizoram, collections were made in Aizawl district covering Aizawl (743 m – 965 m asl), Rungdil (332 m asl), Sairang (50 m – 80 m asl), Sihhmui (184 m asl), Tamdil (745 m asl), Durlui (103 m asl), Tuivawl (488 m asl), Tuirial (179 m asl) and Tuirini (298 m asl); Champhai district including Champhai (951 m – 1460 m asl), Tuichang (603 m asl), Tuipui (708 m asl), Khawzawl (1158 m – 1257 m asl), Kawlkulh (965 m asl) and Khawai (1458 m asl); Mamit district (Lengpui, 390 m – 400 m asl; Tut, 74 m asl and Zawlnuam, 47 m asl), and Kolasib district (Bilkhawthlir, 65 m asl; Buhchang, 46 m asl; Tuitun, 308 m – 326 m asl and Kawnpui, 910 m asl). The surveyed area in the southern part of Mizoram includes Lunglei district (Theiriat with 1048 m - 1060 m asl, and Ramlaitui, 750 m asl), Lawngtlai district (Lawngtlai town, 847 m asl), Saiha district (Khankawn, 193 m asl and New Latawh, 458 m asl) and Serchhip district (Chhingchhip, 1113 m asl; Mat, 651 m asl and Thenzawl, 741 m – 810 m asl).

Euphlyctis cyanophlyctis was mainly collected from the littoral part of lentic and lotic habitats, water canals and temporary pools from the thirty (30) surveyed

sites with an altitude ranging from 46 m – 1460 m asl throughout the year. *Hylarana nicobariensis* was encountered and collected from a wide variety of habitats, from running waters and their adjacent water bodies, to rice paddy fields, to leaf-litters of forest floors, disturbed environment from the twenty six (26) surveyed sites with the elevation ranging from 46 m to 1460 m asl during the months of September to April.

Kaloula pulchra was mainly encountered and collected from their breeding ground, like rock-pools and other temporary pools adjacent to anthropogenic area and forest or forest-edge in the sixteen (16) surveyed areas with altitude ranging from 46 m – 1460 m asl especially during the months of February to June. *Microhyla berdmorei* was encountered in the vegetated lentic pools in or adjacent to the forest stream and river in the twenty one (21) surveyed sites with altitudinal distribution from 46 m asl to 1460 m asl during the months of October to March.

The present observation reported that unlike *Hylarana nicobariensis*, the other three species i.e. *Euphlyctis cyanophlyctis*, *Kaloula pulchra* and *Microhyla berdmorei* avoid the mainstream by retiring to the ephemeral pools and puddles along the course of water channel. It was suggested that these species may diverge to avoid competition, reducing overlap in resource use. Selection of understory vegetation in the riparian zone by the two microhylids (*Kaloula pulchra* and *Microhyla berdmorei*) showed that they are highly selective in microhabitat choice that provides frogs with moisture, shelter, calling and oviposition sites. Both ranids (*Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*) showed their wide distribution throughout the state from lower to higher elevations with different geographic locations that might be due to non-specific in their choice of

microhabitat, and species with broad niches are expected to be more widespread because they may tolerate a greater variety of habitat conditions

The breeding behavior of *Euphlyctis cyanophlyctis* was observed from two study sites namely, study site I consisting of three permanent ponds at Kawnpui and study site IV at Tlawng river in Mizoram. Observations in the field during the study period indicated that the breeding season of *Euphlyctis cyanophlyctis* starts from March which coincides with the onset of monsoon and continued till early August where air temperature recorded was 18 °C – 33 °C, relative humidity between 44.5% - 97% and rainfall between 266 mm – 600.1 mm, water temperature 19.5°C – 29°C and pH of water 6.5 - 7.1. ANOVA test between breeding season and environmental factors (except water pH at the study site-IV, where p>0.05) showed significantly different. By contrast, no significant relationship was detected between breeding season and other ecological factors like pH of water and relative humidity (p>0.05). It was observed that the male by floating on the water surface start the advertisement calls from around 3:30 PM which attract the receptive female towards the breeding site. Advertisement calls lasted for about 524.5 - 683.4 ms emitted at an interval of 0.802 - 1.650 s, each call is composed of 5-12 pulses. Each pulse lasted for 18 – 56.125 ms. The frequency spectra ranged between 1140 – 1609 Hz. Amplexus was axillary where the male grasps the female at the axilla. Males are usually smaller in size than female, and vocal slits are present in male. The Snout Vent Length (SVL) of amplexing females ranges from 31.23 mm - 61.15 mm whereas the SVL of males ranges from 32.20 mm - 46.80 mm. During the breeding season, males are usually with a weakly developed nuptial pad at the base of the first finger. Moreover, the venter in female is usually marbled while it is white in male. During spawning the

eggs were left among aquatic vegetations and slowly sink to the water bottom. The clutch sizes recorded ranged from 68 – 182 and no correlation between the female sizes and the clutch sizes was recorded.

The present observations indicated that *Hylarana nicobariensis* was a seasonal stream-breeder and the breeding takes place from October to February, sometimes even up to March depending on the timing of monsoon which coincide with late autumn to the whole winter season in Mizoram. During the breeding season, the range of air temperature was between 10°C and 35°C, rainfall 0 mm - 269.3 mm, and relative humidity 42% - 96%, water temperature 12°C - 24.5°C and pH of water between 6.5 to 7.4, and the peak period of abundance was December when the water became very shallow with minimum velocity. From the present data, ANOVA test on the breeding season against environmental factors like air temperature, relative humidity, rainfall, water temperature and pH of water (except water pH at the study site-IV, where $p>0.05$) differed significantly. From around 4:30 PM in the evening, males mating vocalizations were heard from different microhabitats like fissures of soil and rock, pebbles and boulders near, amongst the plant debris and aquatic plants. The call consisted of 5 - 9 notes emitted at an interval of 0. 628 - 1.645 s, each note ranged from 0.141-0.165 s with a series of 35 to 47 pulses. A single call lasted for about 1.232 – 2.682 s. The frequency spectra ranged between 2109 – 2296 Hz with a dominant band at 2265 Hz. Mating was solitary and the axillary amplexing pairs floats on water or perch on the substrate until eggs were laid. Distinct sexual size dimorphism was observed in this species where females were larger (with SVL ranged from 42. 36 mm - 58.6 mm) than males (with SVL from 32.34 mm - 48.48 mm), and males are with darker throat and oval flat gland on

the inner arm. Generally, the eggs were deposited among water substrata where water current was very slow. During the present investigation, many clutches of egg even up to 27 clutches were seen adhered with each other in the same breeding site along the rivers or streams which was also observed among a common brown frog, *Rana japonica*. These communal egg masses may provide warmer temperature, protects developing embryos from predators and water current. The clutch sizes recorded ranged from 131 – 628. There was no correlation between female sizes and clutch sizes.

It can be mentioned that the breeding activity of *Kaloula pulchra* in Mizoram was provoked by the first shower of monsoon rain during the month of February or March. From the present observation, the breeding season of *Kaloula pulchra* in Mizoram was very short which start from the month of late February to May, where the atmospheric temperature ranged between 14°C to 33°C, relative humidity fluctuates between 47% - 92% and rainfall between 3 mm - 539.7 mm, water temperature 14.5°C to 28.5°C and pH of water between 6.2 - 6.7 at study sites III and V. ANOVA test breeding season against environmental factors (air temperature, relative humidity, rainfall, water temperature and pH) did not differ significantly, where $p>0.05$. At the onset of dusk around 4:30 PM, males first enter the breeding site and float in the pools of breeding ground, blow up their bodies to make calls. The call consisted of a single note emitted at an interval of 2.876 - 3.902 s, the note repetition rate ranged from 0.21 - 0.25 notes per second. The notes lasted for 450.8 - 620.3 ms and were composed of a series of 36-45 pulses. The frequency spectra had a dominant band at 1265 Hz. After entering the breeding ground, the female approached the calling male from the back and with the help of her fore-limbs she

started tackling the hind-limbs and flanks of the calling male. Suddenly, the male turned back and mounted on the back of the female and amplexing was axillary. Within 20 minutes to 1 hr, female laid and left a raft of eggs floating on the water surface. The clutch size varied from 363 – 576 and SVL of males ranged from 57.74 mm - 69.76 mm and females ranged from 59.90 mm - 69.94 mm. No correlation was observed between clutch sizes and female sizes.

The breeding period of *Microhyla berdmorei* started from October and continued till the month of March where the atmospheric air temperature ranged between 10°C – 33.5°C, rain fall from 0 mm - 269.3 mm and relative humidity 41% - 96%, water temperature 12°C – 23.5°C, and pH of water fluctuates between 6.3 and 7.4. Results on the ANOVA test showed that the breeding season against ecological factors like air temperature, relative humidity, rainfall and water temperature differed significantly, where $p < 0.05$. But the breeding season against pH of water did not differ significantly ($p > 0.05$). During the last part of monsoon season, i.e. late September when the rivers and streams started receding, male mating calls of *Microhyla berdmorei* started around 4:30 PM from secluded spots under pebbles, debris of twigs and small shrubs, and fissures of soil in the breeding area. The call consisted of a single to seven notes emitted at an interval of 3 -5 s, a single call lasted for 1.4 -1.6 s. Each note consisted of 7 – 12 pulses, lasted for 0.2 s at an interval of 0.06 s. The frequency spectra ranged between 1484 Hz – 2046 Hz with dominant frequency 1677 Hz. At around 8:00 PM to 10:00 PM, the female entered the breeding ground and contacted the male. The male clasped the female in axillary amplexus that may last for 2 – 3 hrs or even longer periods. After laying a floating raft of pigmented eggs, the female left the breeding site first then

followed by the male. A raft of eggs clutch was allowed to float and develop by their own usually under the shady lentic habitats. Clutch sizes ranged between 218 and 443. No distinct sexual dimorphism was observed in this species except the females were slightly larger (SVL range between 31.02 mm - 34.35 mm) than the males (SVL between 28.45 mm - 33.88 mm), and males were usually with darker venter and cross fold on the neck. No correlation between clutch sizes and female sizes was recorded in this present observation.

It was confirmed that among the four species, the breeding season of *Euphlyctis cyanophycitis* and *Kaloula pulchra* coincided with early rainy season, whereas the other two species, *Hylarana nicobariensis* and *Microhyla berdmorei* can be regarded as winter breeders as their breeding periods coincided with winter season in Mizoram. During the present investigation, no sexual combat was observed among the four species. From the data of advertisement call analysis, it was found that anuran calls are species-specific. Amplexing was axillary in all the four species. No correlation between female size and clutch size as well as parental care was observed in all the four species.

The histological study on the testis of each species revealed the presence of different developmental stages of spermatocytes during the breeding season. And 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.

The duration for the development and completion of life cycle in *Euphlyctis cyanophycitis* was 64 - 65 days, *Hylarana nicobariensis* 74 - 75 days, *Kaloula*

pulchra 47 days and *Microhyla berdmorei* 109 days. This shows that difference in the duration of embryonic and larval period may also be depending on species. Hatching stages for the embryo of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei* were stages 23, 21, 20 and 19, respectively. In all the four species, maximum length of the tadpoles were seen at stage 40 measuring 64.60 ± 6.64 mm in *Euphlyctis cyanophlyctis*, 40.89 ± 0.15 mm in *Hylarana nicobariensis*, 29.87 ± 1.26 mm in *Kaloula pulchra*, and 24.88 ± 1.73 mm in *Microhyla berdmorei*. The froglets measured 20.24 ± 1.8 mm in *Euphlyctis cyanophlyctis*, 15.5 ± 0.21 mm in *Hylarana nicobariensis*, 9.07 ± 0.47 mm in *Kaloula pulchra*, and 8.21 ± 0.57 mm in *Microhyla berdmorei*. The faster developmental rate may be due to high concentration of nutrient-rich blue-green algae (e.g. *Anabaena*, *Nostoc* and *Oscillatoria*) and Bacillariophyceae in the ephemeral ponds in which the embryonic and larval development takes place. It is also suggested that this enabled them to avoid desiccation by completing their life cycle within a very short period as the hydroperiod of the microhabitats are usually rain-fed pools.

The water temperature for the completion of life cycle in *Euphlyctis cyanophlyctis* under natural and laboratory conditions ranged from $18^\circ\text{C} - 32.5^\circ\text{C}$, for *Kaloula pulchra* from $14.5^\circ\text{C} - 29^\circ\text{C}$, *Hylarana nicobariensis* from $12^\circ\text{C} - 25^\circ\text{C}$ and *Microhyla berdmorei* from $12^\circ\text{C} - 24.5^\circ\text{C}$ that might be the ambient temperature for each species. pH of water for the development of *Euphlyctis cyanophlyctis* in the natural and laboratory showed the highest range i.e., from 6.4 – 7.6 followed by *Hylarana nicobariensis* and *Microhyla berdmorei* (6.3 – 7.4), and *Kaloula pulchra* (6.0 – 6.7). Each pH range was suggested as the optimal pH for the development of each species.

Observation on the results showed that tadpoles of all the four species start feeding from stage 25 onwards. During the early stages of feeding, they feed mostly on detritus and plant materials and during the later stages of feeding they consumed both phytoplankton and zooplankton. The larvae of both *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis* can be put under Orton's type 4 (facultative suspension-feeders), the generalised pond (nektonic) type, while the larvae of *Kaloula pulchra* and *Microhyla berdmorei* are Orton's type 2 (Obligate suspension-feeders), nektonic filter-feeders. Analysis of the intestinal contents revealed that Bacillariophyceae and Chlorophyceae were the main dominant food items in the tadpoles of *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis* and *Microhyla berdmorei*, while Cyanophyceae and Bacillariophyceae were dominant in *Kaloula pulchra*. During the study period, larvivorous behavior was observed among the tadpoles of *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*, but cannibalism was not found among all the four species. Tadpoles soon stop feeding at stage 42 and after metamorphosis the froglet start feeding on a carnivorous diet.

The stomach contents of both adults *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis* were more or less similar and it also shows that they shared common in feeding habitats. Though the two microhylids were found to feed mostly on the same orders of insects as found in ranids, but the items are comparatively smaller sizes. Small group of Isopterans (winged and wingless termites) comprised the highest fraction in the diet of *Kaloula pulchra*, whereas those of Hymenopterans (especially ants) were most common among the food composition of *Microhyla berdmorei*. Present observation on the foods of adult *Kaloula pulchra* and *Microhyla berdmorei* shows that, although microhylids are ant specialist, not only ants and

termites are the prey they consumed, but also small beetles, dipterans, small orthopterans and so on. Current results on the comparatively smaller size foods among adult microhylids might be reflected by their head shape, which is important in the number and size of prey they consumed. Besides other non-insect food items, pieces of plants materials were found in the gut of all the four species.

The oral structures of the tadpoles of *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*, when observed under the stereoscopic binocular microscope, revealed that the oral discs were large, located anteroventrally, not laterally emarginated and directed ventrally. The keratodont starts to appear from stage 25. In both the species, the labial tooth row formula (LTRF), 1/2 in the case of *Euphlyctis cyanophlyctis* and 2(2)/3(1) in *Hylarana nicobariensis* were consistent throughout their larval periods. 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 on tadpoles of *Euphlyctis cyanophlyctis* and *Hylarana nicobariensis* revealed that the oral disc is composed of anterior (upper) labium and posterior (lower) labium. Marginal papillae are present on the edge of the oral disc with a wide dorsal gap, sub marginal papillae are also present. The upper jaw sheath (UJS) is straight with the suprarostrodonts which appeared to be serrated and pointed while the lower jaw sheath (LJS) possesses a central V-shaped groove with the infrarostrodonts which are serrated and blunt, and the mouth is present in between. The tooth rows are uniserial and the labial tooth is keratinized. In *Euphlyctis cyanophlyctis*, teeth are smooth, without indentation but scythe-shaped, and no keratinized spurs (or cusps). Each keratinized labial tooth of both the species is derived from cells in the base of

the tooth ridge and consists of three indistinct regions; a distal head (with around 8 - 12 terminal cusps in *Hylarana nicobariensis*), 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. In case of the two microhylids (*Kaloula pulchra* and *Microhyla berdmorei*), the tadpoles were devoid of keratinized structures, such as jaws and labial teeth, and no LTRF was observed throughout their larval period. In addition, they have a terminally-oriented, as opposed to antero-ventrally oriented mouth which is more convenient for filter feeding.

The present investigation showed that the tadpole intestine of all the four species consists of two spiral coils: the outer coil (duodenum and anterior ileum) reverses direction at the switchback point followed by the inner coil (posterior ileum and colon), which terminates at the rectum. The length of intestine increased from stage 25 to stage 40. Significant positive correlation between the tadpole length and the gut length was observed in all the four species, where $p < 0.01$ at the 0.01 level (2-tailed). The maximum length of intestine was observed at stage 40 where the tadpole reached a maximum total length. From stage 40 to stage 46, the lengths of total body and intestines greatly reduced, showing positive correlation at the 0.05 level. Shortening of the intestine (by 85%, 83%, 80% and 82% in *Euphlyctis cyanophlyctis*, *Hylarana nicobariensis*, *Kaloula pulchra* and *Microhyla berdmorei*, respectively) during spontaneous metamorphosis accompanied by a change in cross-sectional morphology from a single layer of cuboidal epithelial cells into a complicated layers consisting of secondary epithelium, intestinal fold lined with

numerous microvilli were observed in all the four species. The intestine at stage 46 resembles that of the adult and it was found that the aquatic herbivore tadpole is already transformed in to a terrestrial carnivore adult.

Therefore, the present investigation conducted in Mizoram, North East India, contribute more and new informations on the distribution, breeding behavior, habit and habitat, development and food and feeding behavior of ranids (*Euphlyctis cyanophlyctis* and *Hylarana nicobariensis*) and microhylids (*Kaloula pulchra* and *Microhyla berdmorei*) which are prevalent in Mizoram.

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Published papers:

- i) **H. T. Lalremsanga**, Saipari Sailo and R. N. K. Hooroo (2007). Record of *Rana chloronota* (Gunther, 1875) from Mizoram, north-eastern India. *Hamadryad* 31(2): 361-362.
- ii) Saipari Sailo, **H. T. Lalremsanga** and R. N. K. Hooroo (2007). First report of *Amolops kaulbacki* from India. *Herpetological Review*. 38(1): 96.
- iii) **H. T. Lalremsanga**, Saipari Sailo and R. N. K. Hooroo (2007). Record of *Leptobrachium smithi*, a new state report from Mizoram, India. *Herpetological Review*. 38(1): 98.
- iv) **H. T. Lalremsanga**, Saipari Sailo and R. N. K. Hooroo (2007). Record of *Sylvirana leptoglossa* (Cope, 1868) from Kolasib District, Mizoram, north-east India. *Frogleg* 13: 9-10.
- v) H. Vanlaltana, K. Vanlalhraia, T. Lalruatkima, R. Lalthakima, P. C. Chhunthangpuia, Esther Lalhmingliani and **H. T. Lalremsanga** (2007). Studies on the plankton community of Rih Dil, Myanmar. *Science Vision* 7(4): 144-151.
- vi) Lalnunsanga, C. Lalrinchhana, T. C. Laltanpuia, Lalrotluanga, **H. T. Lalremsanga**, Saipari Sailo and C. Laltleipui (2008). Anuran fauna of Mizoram University Campus, Tanhril, Aizawl with notes on their IUCN Criteria and Distribution. *Science Vision* 8(1-2): 13-23.
- vii) T. C. Laltanpuia, C. Lalrinchhana, Lalnunsanga, Lalrothuanga, Ricky Hmingthansanga, Arti Kumari, Vanlalsawmi Renthlei, S. Lalrintluangi and **H. T. Lalremsanga** (2008). Snakes of Mizoram University Campus, Tanhril, Aizawl with notes on their identification keys. *Science Vision* 8(4): 112-127.
- viii) **H. T. Lalremsanga**, Saipari Sailo and R. N. K. Hooroo (2009). Studies on the ecology, breeding behavior and development of *Kaloula pulchra* (Gray, 1831) (Anura: Microhylidae) in Mizoram, Northeast India. *Abstract in 96th Indian Science Congress*. pp.7-8

- ix) Saipari Sailo, **H. T. Lalremsanga**, R. N. K. Hooroo, Lalrotluanga and A. Ohler (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.
- x) C. Lalrinchhana, Lalnunsanga, T. C. Laltanpuia, Arti Kumari, Vanlalsawmi Renthlei, S. Lalrintluangi, Lalrotluanga, Jerry Ramheriana and **H. T. Lalremsanga** (2009). Collections on the Saurian (Reptilia: Squamata) fauna around Aizawl City area with notes on their ecology. *Science Vision* 9(2): 57-72.
- xi) S. Sengupta, Saipari Sailo, **H.T.Lalremsanga**, A. Das, and I. Das (2010). A new species of *Leptolalax* (Anura: Megophryidae) from Mizoram, North-eastern India. *Zootaxa* 2406: 57 – 68.
- xii) **H.T.Lalremsanga**, Lalmalsawma Khawlhring and Lalrotluanga (2010). Three additional lizard (Squamata: Sauria) records for Mizoram, India. *Journal of Threatened Taxa* 2(2): 718-720.

Papers Presentation at Symposia / Seminars /Workshop / Conferences :

- (i) Presented paper entitled ‘Studies on the breeding behaviour and development of *Rana nicobariensis* (Stoliczka, 1970) (Anura: Ranidae)’ in the Regional Symposium on Research Thrust in Animal Sciences in N. E. region- An appraisal (March 24th to 25th, 2006) organized by the Department of Zoology,NEHU,Shillong-22,Meghalaya.
- (ii) Presented paper entitled ‘Studies on the normal development of *Microhyla berdmorei* (Blyth,1856) (Amphibia: Anura)’ and ‘Amphibian Diversity in Mizoram’ in the National Symposium on Advances in Zoology: Faunal Diversity and Ecophysiology (March 13th to 14th, 2008) organized by the Department of Zoology,NEHU,Shillong-22,Meghalaya.
- (iii) Presented paper entitled ‘Preliminary Survey on the Herpetofauna of Mizoram’ in the State level Symposium on Recent Development in Science and Technology (30th May, 2008) organized by Mizoram Science Society in collaboration with Technology and Environment, Govt. of Mizoram.
- (iv) Presented paper entitled ‘Studies on the ecology, breeding and development of *Microhyla berdmorei* (Blyth,1856) (Amphibia: Anura) Mizoram’ in the

SERC 2nd School in Herpetology (1st to 14th Sept 2008) organized by Wildlife Institute of India (WII), Dehradun, India.

(v) Presented paper entitled '**The Biology of Winter-breeding frog in Mizoram, Northeast India**' at the **State Level Symposium on Recent Advances in Science and Technology (2nd December 2009)** organized by Mizoram Science Society in collaboration with Science, Technology and Environment, Govt. of Mizoram held at Millenium Centre, Aizawl.

(vi) Presented paper entitled '**Reptile Diversity in Mizoram**' at the **International Biodiversity Day 2010 on 22nd May 2010** organized by Environment and Forest Department, Govt. of Mizoram at Pachhunga University College.

(vii) Presented paper entitled '**Faunal Diversity Management**' at the **State Level Sensitization Workshop on Biodiversity (21st October 2010)** organized by Mizoram Biodiversity Board (MBB) and Mizo Post Graduate Science Society (MIPOGRASS) held at Chanmari YMA Hall, Aizawl.

Symposia / Seminars / Training / Workshop / Conferences Participated:

(i) **International Workshop of CONSERVATION BEYOND BOUNDARIES, A Training Programme for Conservation Biologists (As Part of Manas' Centenary Celebrations) at Manas National Park, Assam from 23rd to 27th February 2006.** Conducted by Dr. Rosemary Trevelyan, Director, Tropical Biology Association, Department of Zoology, University of Cambridge, UK in collaboration with Aaranyak, Bodoland Territorial Council and Dept. of Environment and Forest, Govt. of Assam.

(ii) **Regional Symposium on Research Thrust in Animal Sciences in N. E. region- An appraisal (March 24th to 25th, 2006)** organized by the Department of Zoology, NEHU, SAP & COSIST (UGC) and DST-FIST.

(iii) **National Symposium on Advances in Zoology: Faunal Diversity and Ecophysiology (March 13th to 14th, 2008)** organized by the Department of Zoology, NEHU and DSA (UGC-SAP) Programme, CSIR, New Delhi.

- (iv) **State level Symposium on Recent Development in Science and Technology (30th May, 2008)** organized by Mizoram Science Society in collaboration with Technology and Environment, Govt. of Mizoram.
- (v) **SERC 2nd School in Herpetology (1st to 14th Sept 2008)** organized by Wildlife Institute of India (WII), SERC and DST.
- (vi) **Workshop on Statistical methods in Medical and Health Sciences (19th to 21st Feb 2009)** organized by Department of Statistics, NEHU and Indian Statistical Institute (ISI), Kolkata.
- (vii) **National Level Technical Workshop on Remote Sensing, GIS and GPS Application in Natural Resource Management (9th to 10th March 2010)** sponsored by North Eastern Council, Shillong and Mizoram University, Aizawl.
- (viii) **Orientation Programme on “Environment and Life Sciences” (5th to 31st October, 2009)** organized by UGC sponsored Academic Staff College, Mizoram University.
- (ix) **Refresher Course in Life Science (9th September to 1st October 2010)** organized by UGC sponsored Academic Staff College, Mizoram University.