STUDIES ON THE ECOLOGY AND BIOLOGY OF
*Rana alticola* (BOULANGER)

ABSTRACT

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The thesis incorporates “Studies on the ecology and biology of *Rana alticola* (Boulenger)” *Rana alticola* falls under the order anura and belong to the family Ranidae. Ranidae are also known as true frogs, typically, are smooth, moist-skinned frogs, with large, powerful legs and extensively webbed feet and many of the true frogs are aquatic or live close to water. *Rana alticola* was originally recorded from Khasi Hills, Meghalaya by (Boulenger, 1882). Pillai and Chanda (1979) recorded the species for the second time from Meghalaya following its first record by Boulenger in 1882. In North East India, *Rana alticola* has been reported from Meghalaya, Assam, Nagaland, Mizoram and Tripura. Besides these, the distribution of *Rana alticola* in India includes West Bengal, Orissa and Andaman. Outside India, it has been reported from Sri Lanka, Nepal, China, Japan, Indonesia, Bangladesh, Myanmar, Thailand and Malaysia. The adults of *Rana alticola* are extremely rare and nocturnal but their tadpoles are abundantly available (Chanda, 1994). Sahu and Khare (1980) published field key of *Rana alticola* tadpoles and also on the food and feeding habits of *Rana alticola* during different stages of metamorphosis (Sahu and Khare 1988) and Grojean et al., (2003) gave the morphology and buccopharyngeal anatomy of the tadpole of *Rana (Nasirana) alticola*. It may be mentioned that Pawar and Birand (2001) reported the presence of *Rana alticola* from Ngengpui Wildlife Sanctuary situated in the Saiha District of Mizoram, North East India, and Sen (2004) also reported its occurrence in Mizoram without mentioning the specific area of collection from the state. Other than these works, little is known about the ecology and biology of the species. However, as with most families of frogs, there is large
variation of habitat within the family. Studies regarding its distribution, habit and habitat, breeding behaviour, development and food and feeding behaviour of *Rana alticola* in Mizoram or other parts of adjoining areas in North Eastern India and from other parts of the globe are not available. Therefore, it is envisaged to take up a study on these aspects to know more about the distribution, habit and habitat, breeding behaviour, development, food and feeding behaviour of *Rana alticola* in Mizoram.

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

1. Study of the Distribution, habit and habitat, food and feeding behaviour and abiotic factors such as temperature, rainfall, pH and humidity in Mizoram.

2. Study of breeding behavior, spawning and nesting.

3. Study of the developmental stages with reference to morphometric changes of the tadpoles.

**NOMENCLATURE OF THE SPECIES**

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

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

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

Chapter III: Development of *Rana alticola*.

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

Conclusion

References

The observations and results of the studies on *Rana alticola* are given chapter wise as follows:-

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

To know the distribution of *Rana alticola* in Mizoram, North East India, extensive survey was conducted from the year 2005 to 2007. Mizoram, situated in the southernmost state in North East India, is one of the mega Biodiversity hotspots of the world and lies between 21°56'N – 24°31’N and 92°16'E – 93°26' E. Mizoram is bounded by the state of Manipur and the Cachar District of Assam to its north, the Chin Hills of Myanmar to its east, by the Arakan Hill ranges of Myanmar to the South and by Tripura State and the Chittagong Hills of Bangladesh on the West. The state is divided into eight districts – Aizawl, Kolasib, Champhai, Mamit, Serchhip, Lunglei, Lawngtlai and Saiha. All the eight districts were surveyed for studying the distribution of *Rana alticola*. Standard survey techniques for amphibians including anuran calling surveys, egg mass surveys, larval surveys, and visual encounter surveys for adults were used. The adults were captured by hand or net and
photographed in live condition. After taking photographs and identification, some of the animals were released back to their natural environment and some were preserved in 4% formaldehyde for further studies. Eggs and tadpoles collected from their natural environment were reared to metamorphosis for species confirmation by maintaining in a plastic tray containing stream or pond water in the laboratory condition. Collections were made from different habitats such as forest covers, rivers, streams, ponds, rain fed pools, open fields with vegetation, etc. Morphometric measurements largely follow the combination of Chanda (1994), Bain et al., (2006) and Ohler (2007).

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

From the above mentioned survey sites, tadpoles were collected from all the 20 sites which are Aizawl, Rung dil, Sairang, Sihhmui, Tam dil, Tuirial, Tuirini, Buhchang, Herhse, Kawnpui, Lun dil, Lengpui, Tut, Chhingchhip, Mat, Thenzawl, Theiriat, Lawngtlai, Khankawn and New Latawh but adults were encountered from 12 survey sites namely Rungdil, Sairang, Tamdil, Tuirini, Herhse, Buhchang, Lundil, Tut, Chhingchhip, Theiriat, Lawngtlai, and New Latawh. It may be suggested that the tadpoles can adapt to different habitat like rivers, streams and pools. During the survey, tadpoles of *Rana alticola* were encountered during the months of August, September, October, November, December, January, February and March. The froglets were found in plenty during the months of April and May. No tadpoles were found in the months of May, June and July indicating that the tadpoles have metamorphosed. The tadpoles were usually found to inhabit a part of water where the current is slow. The tadpoles were usually found in large number.

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

The breeding behavior of *Rana alticola* was observed from three study sites namely Sairang River (Study site I), Herhse stream (Study site II) and Tamdil Lake (Study site III) in Mizoram. Habitat of the three study sites were conducted to record the topography, vegetation, and ecological factors; rainfall, relative humidity, air temperature, water temperature and pH. To determine the breeding period, observations were carried out both in the natural and laboratory condition. In the natural condition, observation was carried out with the help of photography. Audio Encounter Surveys (AES) and Acoustic Encounter Surveys (AES) were used to
identify the locations of the calling males. The calls were recorded with the help of a digital voice recorder model Samsung SVR 380 and transferred to PC and analyzed with the software ‘sound ruler Acoustic Analysis Version 0.9.6.0. Individuals were captured by hand or net. Presence of gravid females and eggs at the study sites gives evidence that it is the breeding season.

Observations in the field during the study period indicated that the breeding season of *Rana alticola* starts from late June which coincide with the rainy season and continue till early October. During the breeding season, the water level at the three study sites increases resulting in strong water currents which in turn carry away the eggs oviposited by the frog around the breeding sites. This may be the reason why tadpoles of *Rana alticola* are found in almost every water bodies whereas the adults are very rare. It was observed that the male emerged first and reaches the breeding site first, and then they start the advertisement calls which attract the receptive female towards the breeding site. During the breeding season, the males of *Rana alticola* started calling from 1400 hours till 2100 hours from the month of June following the emergence. The call sounds like the chirping of bird with a ‘chirp’ sound. The advertisement call is species specific. The advertisement call consisted of four notes and the call duration lasted for one second. Each note consists of two to three pulses and each note lasts for 0.1 second. The advertisement call is the major factor in the courtship of *Rana alticola* which initiate the female to approach the calling male, the male will grasp the female by the armpit and amplexus will start. Amplexus is axillary where the male grasps the female at the axilla. *Rana alticola* also gives a distress call when in captivity which differ from the advertisement call. The distress call consisted of two notes and the call duration lasted for two seconds.
Each note consists of six pulses and each note lasts for 0.3 second. This loud, explosive distress calls is given in response to acute disturbance or grasping by a potential predator and is produced by either sex or sometimes even newly metamorphosed young and are acoustically dissimilar to the advertisement calls.

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

Oviposition sites of *Rana alticola* were found amongst leaf litter along the edge of the water bodies. The eggs were deposited in the stagnant water as well as flowing water but attached to vegetation and were always submerged in the water. The female starts depositing the eggs after some hours from the time of amplexus and amplexus can continue up to 24 hours. The eggs were deposited in multiple clutches and the colour of the egg is light brown. The egg measures about 1.2 to 1.5 mm in diameter. The clutch size ranges from 1002 – 2018 numbers and there is no co relation between SVL of female and the clutch size.
During the non breeding season from the month of November, the adults are very rare in all the three study sites. The adults, if encountered during the non breeding season, were found in bamboo stumps present around the study sites. During this season, there is decrease in the level of the water at all the three study sites and a large number of tadpoles were found during this period. From April to May, only juveniles of *Rana alticola* were seen around the study sites, and no breeding activities were seen during these months. No adults were seen at all but a large number of metamorphosed froglets were observed during these periods. The metamorphosed froglets were seen in a huge number among the vegetations in the water, on bamboo stumps, between rock crevices and on the rock boulders present around the study sites.

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

During the breeding season, i.e., from June to October in all the three study sites, the rainfall ranged from 67 mm to 704 mm. The relative humidity ranged from 24% to 98%. The air temperature recorded was 16°C to 35°C. The water temperature ranged from 13°C to 26°C and pH ranged from 6.5 to 7.5.
Chapter III: Development of *Rana alticola*.

For the purpose of studying the development of *Rana alticola*, amplexing pairs were brought to the laboratory and the time of oviposition was taken as the time of fertilization. Staging of the developmental stages was done on the basis of external morphological changes as per the criteria described by Gosner in 1960. The eggs were maintained at water temperature between 11°C to 26°C in the laboratory which is more or less the temperature in its natural habitat which ranged from 10°C - 25°C at the three study sites. The eggs were carefully observed under a stereoscopic dissecting binocular microscope and the time of onset of each new stage was noted and some eggs from each stage were fixed and preserved for closer examination. Simultaneously, eggs oviposited in the field were also observed in the natural condition where an enclosure was made with the help of a net. Although breeding activities starts from late June, the developmental time in the natural condition (field) was studied from September 2005 to April 2006 and September 2006 to April 2007. During these periods, the level of the water has subsided and the current of the water has also slowed down making it possible to make an enclosure in the field for the study of the development. The study revealed that the complete developmental time of *Rana alticola* takes 215 days which is approximately seven months, which is comparatively long as compared to the developmental time of other ranids. Hatching takes place at stage 19 (i.e. heartbeat stage) after about 13 days and 20 hours, which is relatively late as compared to the other ranids. It is now referred to as hatchling. The operculum closes on day 28 when the hatchling reaches stage 24. From stage 25 onwards, it is referred to as tadpole. The hindlimb buds starts to grow from stage 26 after 51 days from the time of fertilization. The tadpole continues to grow and there
is development of the hind limbs and finally at stage 42, the forelimbs emerged after 194 days and finally complete metamorphosis takes place after 215 days from the time of fertilization. During the period of development i.e from September 2005 to April 2006 and September 2006 to April 2007, in all the three study sites, rainfall ranged from 1.2 mm to 611.4 mm, the relative humidity ranged from 26% to 95%, air temperature ranged from 12°C to 35°C, water temperature ranged from 10°C to 25°C and the pH ranged from 6.4 to 7.6.

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

In order to study the food and feeding habits of *Rana alticola*, different developmental stages of the tadpoles were collected. Soon after collection, the specimens were euthanized in MS-222 and preserved in 4% formaldehyde after which they were autopsied for qualitative analysis of the gut contents. After dissection, the guts were removed and measured with the help of ruler and dial caliper before flushing with distilled water. The gut contents were then taken in a clean slide and qualitative analysis of the gut contents was done under a compound microscope. Identification of the gut contents of the tadpoles and adults were made and photographs of the gut contents were taken with the help of Sony cybershot (5.1 megapixels, DCS-W5) attach binocular (Labomed, CMS-2). Histological changes of the gut of the tadpoles and adult was carried out with the help of a compound microscope. An ultrastructural study of the gut was also done using Transmission Electron Microscopy. The oral structures of the selected developing tadpoles were studied using stereo-scopic binocular microscope following the criteria of Altig and McDiarmid (1999), and also with Scanning Electron Microscopy.
*Rana alticola* belong to the lotic-benthic ecomorphological guild of Altig & Johnston (1989). The oral structures of the tadpole of *Rana alticola*, when observed under the stereoscopic binocular microscope, revealed that, the oral disk is large, anterioventral, not laterally emarginated, directed ventrally. The keratodont starts to appear from stage 25. The mouthpart at stage 25 is simple with labial tooth row formula (LTRF) which is not consistent; and may vary from 1- 2: (2+2) - (4+4) / 1+1: 3-5. The present study established an interesting feature that *Rana alticola* tadpoles have marginal teeth which make their first appearance at stage 26. The number of tooth rows or keratodont rows increases during ontogeny. However, the LTRF at these stages are not consistent and it may vary from 2: (3+3) - (5+5)/ 1+1: 4-7. The LTRF remains the same from stage 26 through stage 41. The oral structure starts to degenerate when the forelimb emerged at stage 42. By the time the tadpole reach stage 43, the labial tooth row has disappeared completely.

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

Qualitative analysis of gut contents of the tadpoles and adults of *Rana alticola* was done and observations revealed that tadpoles started feeding from stage 25 onwards. The gut contents of the tadpoles at stage 25 mainly include phytoplankton like Euglenophyceae, Cyanophyceae, Chlorophyceae and Bacillariophyceae and as the development progresses, the gut contents were mainly zooplankton like Rotifera, Rhizopoda, Cladocera and Copepoda. Cannibalism was observed in the tadpole of *Rana alticola* during the time of food shortage in the laboratory condition. Observation of the gut contents of the adult frog of *Rana alticola* revealed that the diet of the adult frog mainly includes insects. Apart from insects, the diet also includes annelids, crustaceans, fingerlings and some plant materials. Insects include the order Isoptera (e.g. Termites), Lepidoptera (e.g. butterfly), Hymenoptera (e.g. ants), Coleoptera (e.g. beetle), Orthoptera (e.g. grasshopper), Hemiptera and Dipteran flies. On some instances plastic materials, sand particles and pieces of small stones were also found in the gut of the adults.

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

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