Comparative study on the breeding and development of two rhacophorids, *Rhacophorus maximus* Günther, 1858 and *Polypedates teraiensis* (Dubois, 1987)

A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF PHILOSOPHY IN ZOOLOGY

BY

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REGISTRATION NO: MZU/M.Phil./395 of dt.26.5.2017

UNDER THE SUPERVISION OF

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CERTIFICATE

This is to certify that Comparative study on the breeding and development of two rhacophorids, *Rhacophorus maximus* Günther, 1858 and *Polypedates teraiensis* (Dubois, 1987) written by Mercy H. Lalramdinfeli has been written under my supervision.

Shhe has fulfilled all the required norms laid down within the M.Phil. regulations of Mizoram University. The dissertation is the result of her own investigation. Neither the dissertation as a whole nor any part of it was ever submitted by any other University for any degree.

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DECLARATION

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Aizawl: Mizoram

December, 2017.

I, Mercy H. Lalramdinfeli, hereby declare that the subject matter of this dissertation in the record of work done by me, that the contents of this dissertation 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 dissertation has not been submitted by me for any other University or Institute.

This is being submitted to Mizoram University for the degree of Master of Philosophy in Zoology.

(Candidate)

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Dated: (MERCY H. LALRAMDINFELI)

Aizawl, Mizoram

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CHAPTER 1

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 7727 species under 32 families and ca.372 genera are known from the world. Out of 32 families the Rhacophoridae family constitutes a radiation of almost 409 tree frogs and allied species (Frost 2017) with members distributed from Asia to Africa (Duellman, 1999). Although a few groups are primarily terrestrial, rhacophorids are predominantly tree frogs which are arboreal, adapted for life in the trees by having intercalary elements between the terminal and penultimate phalanges, expanded digits disks, and often, extensive webbing on the hands and feet (Duellman and Trueb, 1986).

The Rhacophoridae genus Rhacophorus is distributed in India, Japan, Madagascar, Africa and Southeast Asia. The genus Rhacophorus (Kuhl and Van Hasselt 1822) contains approximately 91 species (Frost, 2017). The genus Polypedates is distributed in East, South and Southeast Asia, and at present known to contain 24 species containing *Polypedates assamensis* (Mathew and Sen, 2009), *Polypedates braueri* (Vogt, 1911), *Polypedates chlorophthalmus* (Das,2005), *Polypedates scolletti* (Boulenger, 1890), *Polypedates cruciger* (Blyth, 1852), *Polypedates discantus* (Rujirawan *et. al.*, 2013), *Polypedates hecticus* (Peters, 1863), *Polypedates impresus* (Yang, 2008), *Polypedates insularis* (Das, 1995), *Polypedates iskandari* (Riyanto *et. al.*, 2011), *Polypedates leucomystax* (Gravenhorst, 1829), *Polypedates macrotis* (Boulenger,1891), *Polypedates maculatus* (Gray,1830), *Polypedates megacephalus* (Hallowell,1861), *Polypedates mutus* (Smith,1940), *Polypedates occidentalis* (Das and Dutta, 2006), *Polypedates otilophus* (Boulenger,1893), *Polypedates pseudocruciger* (Das and Ravichandran, 1998), *Polypedates pseudotilophus* (Matsui *et. al.*, 2014), *Polypedates ranwellai* (Wickramasinghe *et. al.*, 2012), *Polypedates subansiriensis* (Mathew and Sen, 2009) *Polypedates taeniatus* (Boulenger, 1906), *Polypedates teraiensis* (Dubois, 1987) and *Polypedates zed* (Dubois, 1987).

The social behaviors of most anurans are associated with acoustic communication in the form of vocalization (Krishna and Krishna, 2005). Advertisement calls attract females to breeding areas, and announce to other males that a given territory is occupied. Advertisement calls are species specific. Rainfall also fills the pools and ponds and provides excellent breeding

sites for a number of anuran species, as there must be some standing water for their breeding activity. Rainfall may be the primary extrinsic factor controlling the timing of reproductive activity in anurans (Duellman and Trueb, 1986).The diversity of reproductive modes in amphibians is higher than in any other vertebrate group (Caldwell, 1992). Once the males and females have found each other, mating can occur. Fertilization is always external and occurs during amplexus, which can take hours or days depending on the species. The most common and widespread oviposition site of anurans is in free water standing or flowing, permanent or temporary (Duellman and Trueb, 1986). One of the most interesting modes is that of rhacophorids, where majority of them deposit their eggs in foam nests, while others exhibit direct development (Duellman and Trueb, 1994). Their eggs develop into tadpoles and hatch that drop into the water once they reach a certain size (Boulenger, 1903; Duellman and Trueb, 1986; Inger, 1966; Liem, 1970; Orlov *et. al*., 2002). 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). 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. The structures of the mouthparts provides a very useful characteristics feature in the discussion of taxonomical problems and identification of the tadpoles (Altig, 1970, Altig and Pace, 1974, Lee, 1976).The tadpoles than metamorphose into a froglet than into adult frog. Knowledge of food and feeding behavior of tadpoles is very essential as early part of life history of amphibian is dependent on availability of food items in natural habitat (Sinha *et. al.,* 2001).

Rhacophorus maximus commonly known as Nepal flying frog, Günther's tree frog, giant tree frog (Fig. 1.1) is listed as Least Concern by the IUCN Red List due to its extensive distribution and its tolerance of a wide range of habitat types (IUCN *et. al.,* 2006). It was described by Günther in 1858 from the type locality Nepal distributed in southwestern China (Yunnan, Tibet), north eastern India, Nepal, western Thailand, and northern Vietnam, and possibly in Bangladesh. *Polypedates teraiensis* is known as the perching frog, six - lined tree frog, or Terai tree frog (Fig.1.2) which is found in eastern Nepal, eastern, peninsular, and north-Eastern India (West Bengal, Meghalaya, Mizoram, Assam, Arunachal Pradesh, Nagaland, Manipur, Sikkim, also reported for Gujarat and Madhya Pradesh) and Bangladesh, into adjacent Myanmar, and possibly into adjacent China.

Although recent research has been done on the natural history and larval morphology of *Rhacophorus maximus* and *Polypedates teraiensis*, information on the ecology of its habitat, development, feeding and breeding behavior during breeding season in Mizoram remains limited. The knowledge of larval adaptation is essential for adequate amphibian conservation measures. Therefore the present investigation was carried out for a better understanding of its breeding and developmental biology in Mizoram.

Fig. 1.1: *Rhacophorus maximus*

Fig. 1.2: *Polypedates teraiensis*

CHAPTER 2

REVIEW OF LITERATURE

1. Breeding behavior:

Anurans have a biphasic life cycle and they breed in a variety of water bodies such as temporary rainfed ponds, ephemeral pools, cemented tanks and permanent ponds (Khongwir *et. al.,* 2016). Breeding activity of rhacophorids like most anurans are influenced by certain factors like rainfall (Ritke *et. al.,* 1992; Donnelly and Guyer, 1994) and temperature (Briggs, 1987; Fukuyama and Kusano, 1992).

Review of literature reveals that the breeding behavior in relation to mating calls, courtship, sexual dimorphism and spawning of rhacophorids has been done by few workers, Liao W.B., and Lu X., (2010) gave information about the breeding activity and behavior of the Omei tree frog, *Rhacophorus omeimontis* (Stejneger, 1924) in south-west China. Kasuya *et. al.,* (1996) have reported the reproductive behavior of the Japanese Tree frog, *Rhacophorus arboreus* (Okada and Kawano, 1924). Wildenheus *et. al.,* (2010) studied the larval and juvenile stages of *Rhacophorus maximus* Günther, 1858 occurs in Vietnam. Khongwir *et. al.,* (2016) reported the breeding and nesting behaviour of *Rhacophorus maximus* Günther, 1858 at Cherrapunjee and Mawsynram, Meghalaya, North East India. Biju (2009) reported the nesting behaviour of leaf folding of a tree frog, *Rhacophorus lateralis* (Boulenger, 1883) in the Western Ghats, India which is the first report in the family Rhacophoridae, and in the Asiatic amphibians. Chakravarty *et. al.,* (2011) from Assam reported the tadpole morphology and the developmental stages of *Polypedates teraiensis* (Dubois, 1987). Tamuly and Dey (2014) reported the larval morphology and development of *Poypedates teraiensis* (Dubois, 1987) in Assam, North East India. Vassilieva *et. al.,* (2013) report on the reproductive biology of *Rhacophorus vampyrus* (Rowley *et. al.,* 2010) from the Lang Bian Plateau in southern Vietnam and observed that two clutch types: fertilized eggs included in a typical foam nest and unfertilized eggs, apparently having a trophic function, enveloped by dense mucous.

The major factor in anuran courtship is the production of advertisement calls by males. Mating occurs when male frogs make an advertisement calls to attract female frogs for mating and clasped the female frogs at their back sides which results in axillary amplexus in these two rhacophorid frogs, *Rhacophorus maximus* and *Polypedates teraiensis*. Vocalization is the most common method of their social behavior in most anurans (Krishna *et. al.,* 2005). Roy *et. al.,* (2004) have reported the role of weather condition on the daily appearance and the advertisement

call initiation of *Polypedates leucomystax* (Gravenhorst, 1829) during breeding season. Dehling *et. al.*, (2010) have studied the advertisement calls of *Rhacophorus angulirostris* Ahl, 1927 and *Rhacophorus everetti macroscelis* Boulenger, 1896 from Gunnung Kinabalu, Sabah, East Malaysia. Kanamadi *et. al.*, (1993) studied the vocalization of the tree frog *Polypedates maculatus* (Gray, 1830). Gogoi and Sengupta (2017) reported the acoustic characters of four Polypedates species such as *Polypedates himalayensis, Polypedates leucomystax* (Gravenhorst, 1829), *Polypedates teraiensis* (Dubois, 1987) and *Polypedates maculatus* (Gray,1830).

Oviposition site selection is an important factor for the success of anuran amphibians that breed in a wide variety of aquatic habitat as the survival and growth of the offspring that lack parental care may depend on the quality of the habitat (Hooroo *et. al.*, 2017). According to Duellman and Trueb (1986), fertilization occurs at the time of oviposition, and the pair is in amplexus in most anurans and mention that there is some evidence for various kinds of tactile signals between the sexes. Hooroo *et. al.*, (2017) have investigated the oviposition sites of 13 species of anurans, *Amolops assamensis* (Sengupta *et. al.*, 2008), *Euphlyctis cyanophylctis* (Schneider, 1799), *Fejervarya teraiensis* (Dubois, 1984), *Hyla annectans* (Jerdon, 1870), *Kaloula pulchra* (Gray, 1831), *Leptotalax khasiorum* (Das *et. al.*, 2010), *Odorrana livida* (Blyth, 1856), *Odorrana mawphlangensis* (Pillai and Chanda, 1977), *Polypedates himalayensis*, *Polypedates teraiensis* (Dubois,1987), *Rhacophorus bipunctatus* (Ahl, 1927), *Rhacophorus maximus* Günther, 1858 and *Xenophrys parva* (Boulenger,1893) in East Khasi Hills, Meghalaya, North East India. Khongwir *et. al.*, (2016) reported that *Rhacophorus maximus* Günther, 1858 select its oviposition site at the temporary rainfed pond and construct foam nest on diverse substrata in Cherrapunjee and Mawsynram, Meghalaya North East India. Holomuzki (1995) have reported that female American toad, *Bufo americanus* (Holbrook, 1836) often oviposited in fishless, isolated pools disconnected from the stream. Iwai *et. al.*, (2007) studied the choice of ovipostion site selection in Japanese brown frog, *Rana japonica* (Boulenger, 1879) and examined whether the presence and developmental stage of conspecific egg masses affected the choice of oviposition site.

Morphometric measurement:

Several components of the body are defined by the measurements and require clarification (Altig and Mc Diarmid, 1999). Accurate measurement of amphibian morphometrics is essential for taxonomy, studies of growth and development, and studies of fluctuating asymmetry (Browne, 2014). Morphometric measurement is also useful for species description. Watters *et. al.,* (2016) review the morphometric measurements used in anuran species descriptions to rectify the lack of methodological standardization for the measurement of morphological characters in anurans through an extensive literature survey of 136 species descriptions representing 45 recognized families of frogs. Luu *et. al.,* (2014) reported the occurrence of *Rhacophorus maxim*us Günther, 1858 for the first time from Laos and reported the morphological characters of the species. Altig (2007 b) summarize the key measurements and terms and mention that the landmarks used in measurements must be accurately and repeatably located across taxa and stages.

2. Development and metamorphosis:

The life cycle of most anurans undergoes egg laying followed by the hatching of a tadpoles and metamorphosis of tadpoles into a froglet then into the adult frog. For anurans, staging tables are a condensed way of describing ontogenetic changes (Hall *et. al.,* 1997). Therefore recording tables of different development stages for studies of ontogenic changes are very useful for understanding the ecology of species and descriptive of any species. The changing appearance of embryos necessitates a method of quantifying the progress of development. Complete tables of development are necessary for accurate comparison of developmental stages in different organisms (Duellman and Trueb, 1986). Metamorphosis is one of the striking of developmental phenomena, and the extensive morphological changes undergone by some species (Swammerdam, 1737). In Anurans these changes includes the resorption of tail, development of the front and hind limbs and large changes in most organ systems. All the events of larval growth and metamorphosis are controlled ultimately by hormones (Duellman and Trueb, 1986).

Review of literature reveals that there are several reports on the development and metamorphosis of Rhacophoridae species, *Polypedates leucomystax* (Alcala, 1962), *Polypedates teraiensis* (Chakravarty *et. al.,* 2011; Tamuly and Dey, 2014), Kam *et. al.,* (1997) studied the

growth, development and survivorship of *Chirixalus eiffingeri* (Boettger, 1895) tadpoles in 2 bamboo tree habitats at the Experimental Forest of National Taiwan University at Chitou. Das *et. al.,* (2016) reported the morphology and larval development in *Feihyla kajau* (Dring, 1983) from Kubah National Park, Sarawak, East Malaysia (Borneo).

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

Understanding the orientation of the oral discs of a tadpole is an essential component its description and for comprehending the feeding ecology of the species (Mc Diarmid and Altig, 1999). The complex lifecycle of anurans is especially spectacular 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).Cannibalism may be more common than reported, especially among tadpoles of frogs that breed in ephemeral ponds that are subjected to unpredictable drying or where resources shortages and overcrowding may be common and survivorship low (Bragg,1965; Crump,1986; Downie 1990b).Cannibalistic tadpoles most commonly eat conspecifics of different sizes or developmental stages (Hoff *et. al.,*1999). Orton (1953) formalized the concept of ecomorphological diversity of the anuran tadpoles, a scheme was again revised by Mc Diarmid and Altig (1999).

Review of literature reveals that food and feeding behavior in relation to oral apparatus of the tadpoles has been done by few workers, Jenssen (1967) studied the food habits of green frog, *Rana clamitans* (Latreille, 1801), before and during metamorphosis in Southern Illinois. Flores-Nava and Vera-Munoz (1999) reported the feeding behavior of *Rana catesbeiana* tadpoles at different rearing densities. Tamuly and Dey (2014) have studied the external morphology and the oral disc of *Clinotarsus alticola* (Boulenger, 1882) tadpoles from Rosekandy Tea Estate of Cachar District, Assam. Dey and Goswami (2015) described the diet of the tadpoles of *Microhyla ornata* (Duméril and Bibron, 1841) from a tea estate, Cachar, Assam. 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. Devi *et. al.,* (2016) have studied the morphological and micro-structural changes of the oral apparatus in two Anuran tadpoles, *Polypedates teraiensis* (Dubois, 1987) and *Hylarana leptoglossa* (Cope, 1868) in regards to pH. Lalremsanga and Hooroo (2012) have studied the remodeling of the intestine of microhylid frog, *Microhyla berdmorei* (Blyth, 1856) from Kolasib

District and Tlawng river, Mizoram along with the changes in diets between larval stages and adult stages.

CHAPTER 3

OBJECTIVES

The major objectives of the proposed study are as follows:

1. To study the habitat of *Rhacophorus maximus* and *Polypedates teraiensis.*

2. To study the breeding behavior with respect to environmental factors.

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

4. To study oral morphology, food and feeding behavior of tadpoles and adults of *Rhacophorus maximus* and *Polypedates teraiensis.*

CHAPTER 4

MATERIALS AND METHODS

3.1. Study sites

To study the breeding behavior, habit and habitat, and the environmental factors of the breeding sites of *Rhacophorus maximus* and *Polypedates teraiensis*, field survey was conducted from August, 2016 to July, 2017 and two study sites designated as study sites I and II were selected inside the campus of Mizoram University, Tanhril, Aizawl. The characteristic features of the study sites were as follows:

Study Site I: An artificial Pond (circumference = 26.38 m) located near Lianchhiari road (with a GPS location of N 23°44'15.7": E 92°40'02.5" at an elevation of 824 m asl) (Fig. 3.1.1).

Study Site II: An old water storage tank (3.54 m x 2.32 m) located near Lengteng Boys Hostel (with a GPS location of N 23°44'18.0": E 92°39'43.2" at an elevation of 775 m asl) (Fig. 3.1.2).

Fig. 3.1.1: Study site I

Fig. 3.1.2: Study site II

Breeding behavior, amplecting pairs and freshly spawned eggs were studied and documented with the help of photographic and video cameras. The amplecting pairs and their newly laid were photographed with a camera, Sony Cyber-shot DSC-H10 (Super Steady Shot). The temperatures of atmospheric, water and relative humidity of the study sites were recorded by thermometer. Water pH was also measured with the help of pH pen (Hanna instrument).

3.2. Acoustic analysis

Mating calls were recorded with the help of digital voice recorder Sony ICD-PX440 Proffesional compact voice recorder. The sampling used to convert the signals to digital format was 8 KHz with 16–bit precision. The oscillogram was prepared and analyzed with the help of a software tool "SoundRuler Version 0.9.6.0 (acoustic analysis)". The notes are composed of groups of pulses. Notes were 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. (Lalremsanga *et. al.,* 2014). The data were analyzed with the help of statistical software tools SPSS (7.5.1 version) and OriginPro 8 SRO (8.0724 version).

3.3. Development and Metamorphosis

To study the development and metamorphosis of the two species, the investigation was conducted at the two study sites as well as in the laboratory. Amplecting pairs i.e., male and female frogs 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. At the same time, the freshly spawned eggs were also collected. Some egg masses were fixed immediately in the field in 5% formaldehyde. 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 (CETI 9554.1200). 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 embryos and larvae of 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 (CETI 9554.1200) 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.

3.4. Morphometric Measurements

Measurements of the frogs were carried out using a dial caliper accurate to 0.02 mm. Morphometric measurements largely follow the combination of Chanda (1994), Bain *et. al.*, (2006) and Ohler (2007). Abbreviations used are as follows:

SVL: Snout - vent length.

HW: Head width.

HL: Head length.

MN: Distance from the back of mandible to the nostril.

MFE: Distant from the back of the mandible to the front of the eye.

MBE: Distant from the back of the mandible to the back of the eye.

IFE: Distant between the front of the eye.

IBE: Distant between the back of the eye.

IN: Inter nasal space.

EN: Eye to nostril (distance from the front of the eye to the nostril).

EL: Eye length.

SL: Snout length (distance from the front of the eye to the tip of the snout).

SN: Snout to nostril (distance from the nostril to the tip of snout).

TYD: Greatest tympanum diameter.

TYE: Distance from tympanum to the back of eye.

IUE: Minimum distant between upper eyelids.

UEW: Maximum width of inter upper eyelids.

FLL: Fore limb length (from proximal end of arm with to tip of longest finger).

HAL: Hand length (from the base of outer palmar tubercle to tip of finger).

TFL: Third finger length.

PA: Width of pads of fingers.

WA: Width of fingers.

FL: Femur length.

TL: Tibia length.

TFOL: Length of tarsus and foot.

FOL: Foot length.

FTL: Fourth toe length.

PP: Width of pads of toes.

WP: Width of toes.

IMT: Length of inner metatarsal tubercle.

ITL: Inner toe length.

MTTF: Distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between third and fourth toe.

TFTF: Distance from the maximum incurvation of the web between third and fourth toe to the tip of fourth toe.

MTFF: Distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between fourth and fifth toe.

FFTF: Distance from the maximum incurvation of the web between fourth and fifth toe to the tip of fourth toe.

WTF: Webbing between third and fourth toe (from the base of the first subarticular tubercle),

WFF: Webbing between fourth and fifth toe (from the base of the first subarticular tubercle).

T1: From base of foot to tip of longest toe.

T2: From base of foot to tip of second toe.

T3: From base of foot to tip of third toe.

T4: From base of foot to tip of fourth toe.

T5: From base of foot to tip of fifth toe.

Morphometric measurement of the tadpoles were also taken using a dial caliper following the method of Altig (2007). Different stages were categorized into Operculum, oral discs and pigmentation, hind limb bud development, toe differentiation and metamorphic stage as per given by Mc Diarmid and Altig, (1999). Five number of tadpoles (N=5) were measured in different developmental stages. Abbreviations used are as follows:

TL: Total Length

TAL: Tail Length

BL: Body Length

BW: Body Width

IOD: Interorbital Distance

IND: Internarial Distance

SO: Snout - orbit Distance

SN: Snout - naris Distance

MTH: Muscle Tail Length

TMW: Tail Muscle Width

3.5. Food and feeding behaviors in relation to their oral structures.

To study the food and feeding habits of the species, different developmental stages of the tadpoles were collected and preserved in 4% formaldehyde and autopsied for qualitative analysis of the gut contents. The gut of each tadpoles were dissected, the contents were transferred to a watch glass and mixed with 0.5 ml of water. One drop of gut content was placed on a glass slide, covered by a coverslip and were examined under microscope (10x, 40x) following the method done by Dey and Goswami (2008). All food items were expressed in terms of percent abundance and percent frequency of occurrence. The degree of dominance of food items in stomach sample was calculated by Berger- Parker diversity index which is as follows;

$$D = N_{max} / N$$

where, N = the total no. of individuals

N_{max} = the no. of individual in the most abundant resource.

The species diversity was calculated by Shannon – Wiener index

$$H' = -\Sigma\ pi \ln pi$$

where, pi = proportional abundance of the species

$= (ni/N)$.

The species evenness was calculated by

$$E = H' / \ln S$$

where, S = total no. of species.

Identification on the food items of the tadpoles were made following the methods of Edmonson (1959) and Smith (1994). Food items were identified upto genus level. The oral structures of the tadpoles were also studied using stereoscopic binocular microscope (CETI 9554. 1200) following the criteria of Altig and McDiarmid (1999) and Altig (2007). Description of oral disc and labial tooth raw formula (LTRF) were also made according to the method done by Altig (2007). Lengths of the intestines were also measured for different tadpole stages in order to know their rate of shortening of intestines in relation to developing tadpoles.

CHAPTER 5

RESULTS

5.1. Description

1. *Rhacophorus maximus* Günther, 1858:

Morphology: Head broader than long, tympanum rounded, nostril closer to tip of snout, fingers webbed, discs prominent, subarticular tubercules well developed, inner metatarsal tubercule present.

Sexual dimorphism: Females being larger than males. Males with internal vocal sacs.

Coloration: Dorsum in life is uniformly green and venter is fawn, color of dorsum sometimes becomes dark green (Fig. 5.1.1) depending on the species mood and activity in connection with lights and humidity. Dorsum in preservative is blue to violet and venter is light brown.

Habit and Habitat: Nocturnal and arboreal. Inhabit tropical and sub tropical broad leaf forests Retreat in moist crevices or tree holes during non breeding season.

Tadpole Description:

Diagnosis: Body yellow to fawn color, tail yellowish cream with a grey pigmentation fading towards the tip. Body sides and venter are only slightly pigmented. The tail muscle are yellow to fawn color pigmentation fading towards the end of the tail. The intestinal coil is visible with dark brown pigmentation.

Morphology: Body oval – elongated, pointed snout, eyes dorsolateral, nostrils dorsal, open, spiracle sinistral, extended as a short tapered tube, attached to body wall, slightly visible under a microscope. Dorsal and ventral fin transparent, with irregular golden brown patches.

Oral Discs: Oral disc emarginated**,** Mouth ventral, marginal papillae, dorsal gap in marginal papillae, papillae white to transparent, submarginal papillae, jaw sheaths serrated, dark brown, V-shaped lower jaw sheath. LTRF: 5(4-5)/ 3(1).

2. *Polypedates teraiensis* (Dubois, 1987):

Morphology: Head moderate size, wider than long, Snout oval, nostrils rounded, closer to the tip of snout, tympanum rounded, tips of fingers rounded, fingers webbed, discs present, subarticular tubercules prominent and rounded, metatarsal tubercule present.

Sexual dimorphism: Males smaller than females. Males with internal vocal sacs, present on lateral part of buccal floor.

Coloration: Body colors in life are mostly brownish, yellowish, fawn to yellow, brown to fawn with a dark spots and stripes on the back side of the body (Fig. 5.1.2). Color in preservative is brown to fawn in dorsum and venter is white to yellowish with irregular dark patches.

Tadpole Description:

Diagnosis: Body light brown to greyish in color, intestinal coil visible, tail muscle yellow to brown in color fading towards the tip. Black spot present all over the body and tail.

Morphology: Body is of moderate size, head oval, snout slightly rounded and depressed, eyes lateral in position, nostrils dorsal, nares are placed nearer to the snout than to the eye, spiracle sinistral, fin transparent, tail tip greatly narrowing near tip, tail tip pointed.

Oral Discs: Oral disc antero-ventral positioned, emarginated, marginal papillae, submarginal papillae on the lower labium, jaw sheath serrated, lower jaw sheath V-shaped. LTRF: 4(3-4)/3(1).

Fig. 5.1.1: ***Rhacophorus maximus***

Fig. 5.1.2: ***Polypedates teraiensis***

5.2. Morphometric measurements:

The morphometric measurement of the amplecting pairs (males and females) of the two species were also studied. While majority of the frogs were released back, some were 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. Measurement of the amplecting frogs (in mm) were carried out using a dial caliper accurate to 0.02mm.

1. *Rhacophorus maximus* Günther, 1858:

Six of the males and females of *Rhacophorus maximus* were measured. It is found that females are larger (SVL = 70.27 - 76.90 mm) than males (SVL = 47.88 - 70.14 mm). Sexual dimorphism was represented by their sizes.

Table 1: Morphometric measurements of male and female *Rhacophorus maximus*

(N= Total number of frogs examined)

Sl. No.	Characters	Males N=6		Females N=6	
		Range (mm)	Mean ± SE	Range (mm)	Mean ± SE
1.	SVL	47.88 - 70.14	53.23 ± 7.96	66.48 - 76.90	63.26 ± 4.01
2.	HW	15.24 - 24.84	21.35 ± 3.57	20.64 - 27.69	21.73 ± 2.62
3.	HL	15.11- 23.24	17.78 ± 2.98	20.6 - 33.4	22.69 ± 4.49
4.	MN	11.27- 21.42	15.09 ± 3.36	15.53 - 27.79	18.36 ± 4.06
5.	MFE	8.64 -16.42	12.09 ± 2.67	11.54 - 22.99	13.31± 4.19
6.	MBE	2.08 - 7.64	4.68 ± 1.91	6.14 - 8.99	7.89 ± 3.83
7.	IFE	10.59 - 13.67	12.50 ± 1.59	14.02 - 18.74	13.40 ± 1.90
8.	IBE	11.72 - 21.70	17.94 ± 3.69	19.53 - 20.8	18.31 ± 1.87
9.	IN	5.19 - 7.17	5.46 ± 0.86	6.23 - 8.86	6.90 ± 1.10
10.	EN	3.05 - 6.97	4.68 ± 1.29	4.59 - 6.78	4.74 ± 0.86
11.	EL	5.93 - 9.92	6.60 ± 1.56	6.74 - 11.88	7.43 ± 1.88
12.	SN	3.88 - 7.44	4.66 ± 1.26	5.16 - 11.24	5.46 ± 1.26
13.	SL	8.87 - 11.74	8.91 ± 1.07	10.49 - 16.99	11.04 ± 2.44
14.	TYD	3.45 - 4.68	3.85 ± 0.72	4.5 - 5.92	4.46 ± 0.61
15.	TYE	1.01 - 2.81	1.65 ± 0.68	1.42 - 3.9	2.30 ± 0.86
16.	IUE	4.39 - 9.42	6.86 ± 1.79	7.7 - 19.87	9.65 ± 4.65
17.	UEW	4.1- 9.7	5.81 ± 2.25	4.11 - 10.27	5.59 ± 2.20
18.	FLL	12.24 - 18.2	14.14 ± 2.20	10.04 - 19.26	14.46 ± 3.48
19.	HAL	10.07 - 21.34	14.76 ± 3.81	15.73 -19.2	15.00 ± 1.32
20.	TFL	5.9 - 13.64	9.66 ± 3.03	10.47-15.31	11.12 ± 1.73
21.	PA I	1.14 - 3.24	1.78 ± 0.75	1.13 - 2.61	1.70 ± 0.60
22.	PA II	2.72 - 5.24	3.49 ± 0.97	3.56 - 4.9	3.64 ± 0.50

23.	PA III	3.38 - 5.94	3.93 ± 0.86	4.41- 5.99	4.32 ± 0.62
24.	PA IV	3.4 - 5.64	3.63 ± 0.88	4.16 - 5.98	4.48 ± 0.77
25.	WA I	1.02 - 2.72	1.53 ± 0.63	1.32 - 2.89	1.99 ± 0.72
26.	WA II	2.05 - 2.96	2.12 ± 0.39	2.47 - 3.91	2.95 ± 0.60
27.	WA III	2.04 - 3.78	2.42 ± 0.65	2.28 - 4.7	2.95 ± 0.96
28.	WA IV	2.29 - 3.74	2.63 ± 0.55	3.79 - 4.56	3.40 ± 0.31
29.	FL	23.59 - 36.5	28.97 ± 12.91	32.31- 41.2	31.96 ± 2.84
30.	TL	23.78 - 35.06	27.80 ± 4.13	33.31- 41.21	31.63 ± 2.71
31.	TFOL	32.43 - 47.58	35.63 ± 5.71	47.07- 48.82	41.06 ± 0.45
32.	FOL	19.18 - 31.22	23.73 ± 4.69	27.74 - 33.13	26.12 ± 1.99
33.	FTL	14.01- 22.08	15.09 ± 2.77	13.62 - 21.36	16.19 ± 2.69
34.	PP I	1.09 - 3.64	1.86 ± 0.88	2.02 -3.89	2.33 ± 0.69
35.	PP II	2.15 - 4.22	2.52 ± 0.71	2.5 - 3.99	2.84 ± 0.66
36.	PP III	2.01- 4.64	2.73 ± 0.86	3.01- 4.52	3.32 ± 0.55
37.	PP IV	2.85 - 4.72	3.14 ± 0.63	3.48 - 5.88	3.91 ± 1.01
38.	PP V	2.7- 4.98	3.23 ± 0.80	2.57- 5.88	3.49 ± 1.14
39.	WP I	0.87 - 2.66	1.38 ± 0.62	1,47- 2.91	2.02 ± 0.61
40.	WP II	1.5 - 2.84	1.95 ± 0.62	2.05 - 3.52	2.29 ± 0.52
41.	WP III	1.05 - 3.32	2.06 ± 0.79	2.01- 3.88	2.85 ± 0.76
42.	WP IV	1.93 - 3.42	2.16 ± 0.53	2.15- 3.47	4.98 ± 17.45
43.	WP V	1.85 - 3.0	2.25 ± 0.48	2.01- 3.96	2.76 ± 0.89
44.	IMT	1.96 - 4.02	2.65 ± 0.89	2.47-11.32	4.27±3.41
45.	ITL	5.23 - 11.62	7.64 ± 2.64	6.79 - 12.54	8.66 ± 2.00
46.	MTTF	15.03 - 20.49	16.15 ± 2.45	22.25- 26.84	21.21 ± 2.01
47.	TFTF	6.58 - 11.92	7.57 ± 1.83	6.52 - 11.56	7.88 ± 2.00
48.	MTFF	17.77- 26.26	18.92 ± 3.04	7.09 - 28.8	20.63 ± 8.03
49.	FFTF	6.29 - 10.39	7.12 ± 1.31	8.65 - 12.97	9.56 ± 1.43
50.	WTF	6.52-10.95	7.56 ± 1.90	8.35-13.24	9.54 ± 2.45
51.	WFF	7.25 -11.90	8.74 ± 1.81	8.51-13.29	9.89 ± 1.63
52.	T1	5.23- 10.93	7.53 ± 2.07	9.22 - 12.0	9.29 ± 0.96
53.	T2	11.1-15.77	11.98 ± 1.61	14.8 - 19.17	14.30 ± 1.58
54.	T3	18.08 - 23.06	17.99 ± 2.07	18.71- 25.27	20.02 ± 2.27

2. *Polypedates teraiensis* (Dubois, 1987):

Five numbers of the adult males and females of *Polypedates teraiensis* were measured. Females are larger (SVL = 74.58 – 75.22 mm) than males (SVL = 52.93 – 55.82 mm).

Table 2: Morphometric measurements of male and female *Polypedates teraiensis*

(N= Total number of frogs examined)

Sl. No	Character	Males N=5		Females N=5	
		Range (mm)	Mean ± SE	Range (mm)	Mean ± SE
1	SVL	52.93 - 56.45	55.07 ± 1.41	67.89 - 75.22	72.93 ±3.13
2	HW	15.15 - 16.79	15.86 ± 0.61	19.73 - 22.78	21.74 ±1.18
3	HL	22.79 - 24.44	23.67 ± 0.69	28.19 - 31.13	29.64 ±1.21
4	MN	20.14 - 22.45	21.26 ± 0.86	26.39 - 30.33	27.97 ±1.52
5	MFE	15.31 - 16.51	16.12 ± 0.47	20.32 - 23.28	21.75 ±1.37
6	MBE	10.23 -11.81	11.09 ± 0.65	13.06 - 17.81	15.30 ±1.71
7	IFE	10.94 -12.46	11.09 ± 0.22	13.34 - 15.89	15.17 ±1.09
8	IBE	15.9 - 17.15	16.45 ± 0.51	19.65 - 22.90	21.45 ±1.20
9	IN	4.23 - 5.22	4.51 ± 0.41	5.34 - 6.78	6.23 ± 0.55
10	EN	5.34 - 6.77	5.96 ± 0.62	6.31 - 8.16	7.38 ± 0.67
11	EL	6.23 - 6.52	6.33 ± 0.11	6.24 - 7.29	7.03 ± 0.45
12	SN	3.01 - 3.84	3.29 ± 0.34	3.48 - 4.34	3.95 ± 0.32
13	SL	9.01 - 10	9.38 ± 0.38	10.45 - 12.55	11.51 ± 0.79
14	TYD	3.09 - 4.65	3.87 ± 0.73	4.15 - 5.6	5.03 ± 0.60
15	TYE	1.23 - 1.61	1.36 ± 0.16	1.99 - 2.98	2.51 ± 0.42
16	IUE	5.33 - 6.53	6.08 ± 0.46	9.72 - 11.46	10.55 ± 0.66
17	UEW	4.05 - 4.88	4.55 ± 0.42	4.69 - 5.61	5.3 ± 0.36
18	FLL	10.94 - 12.81	11.69 ± 0.72	16.56 - 18.54	17.48 ± 0.74
19	HAL	12.09 - 13.95	12.9 ± 0.93	18.67 - 20.64	20 ± 0.79
20	TFL	8.04 - 8.76	8.44 ± 0.26	10.78 - 12.67	11.74 ± 0.75
21	PA I	1.23 - 1.8	1.58 ± 0.21	2.02 - 2.38	2.22 ± 0.15
22	PA II	2.09 - 2.51	2.33 ± 0.19	2.47 - 3.65	3.11 ± 0.46
23	PA III	2.06 - 2.73	2.33 ± 0.32	3.67 - 4.54	4.09 ± 0.37
24	PA IV	2.03 - 2.82	2.44 ± 0.32	3.89 - 4.76	4.4 ± 0.41
25	WA I	0.12 - 0.92	0.36 ± 0.33	0.77 - 1.23	1.04 ± 0.19
26	WA II	0.01 - 0.96	0.43 ± 0.36	0.93 - 1.96	1.51 ± 0.42
27	WA III	0.06 - 0.89	0.5 ± 0.3	1.04 - 2.34	1.58 ± 0.49
28	WA IV	0.13 - 0.99	0.57 ± 0.31	1.05 - 2.09	1.58 ± 0.45
29	FL	26.49 - 30.67	28.14 ± 1.61	35.55 - 42.61	38.75 ± 2.86

30	TL	28.71 - 30.35	29.27± 0.71	37.67 - 42.24	39.83 ± 1.82
31	TFOL	22.51 - 23.47	22.97 ± 0.37	30.67 - 32.77	31.95 ± 0.92
32	FOL	22.91 - 23.72	23.22 ± 0.35	30.54 - 32.92	32.07 ± 1.04
33	FTL	16.08 - 17.24	16.58 ± 0.47	21.56 - 23.78	23.05 ± 0.92
34	PP I	1.08 - 1.78	1.37 ± 0.26	2.01 - 2.79	2.38 ± 0.34
35	PP II	1.38 - 1.92	1.59 ± 0.22	2.76 - 3.56	3.15 ± 0.29
36	PPIII	1.63 - 2.11	1.82 ± 0.19	2.78 - 3.65	3.21 ± 0.38
37	PPIV	1.8 - 2.05	1.94 ± 0.09	2.99 - 3.78	3.53 ± 0.31
38	PP V	1.63 - 2.17	1.91 ± 0.22	2.87 - 3.35	3.15 ± 0.18
39	WP I	0.11 - 0.8	0.52 ± 0.29	0.92 - 1.43	1.21 ± 0.19
40	WPII	0.23 - 0.89	0.62 ± 0.28	1.04 - 1.61	1.41 ± 0.22
41	WPIII	0.45 - 0.77	0.65 ± 0.15	1.11 - 1.45	1.3 ± 0.12
42	WPIV	0.18 - 0.91	0.66 ± 0.29	1.11 - 1.79	1.46 ± 0.33
43	WPV	0.19 - 0.96	0.67 ± 0.29	1.04 - 1.63	1.42 ± 0.23
44	IMT	1.21 - 1.51	1.33 ± 0.12	1.91 - 2.89	2.6 ± 0.39
45	ITL	6.04 - 6.81	6.46 ± 0.28	7.97 - 10.91	8.46 ± 1.27
46	MTTF	12.98 - 19.43	13.19 ± 0.17	17.39 - 19.42	18.45 ± 0.79
47	TFTF	7.12 - 8.06	7.45 ± 0.37	11.92 - 12.54	12.28 ± 0.22
48	MTFF	14.04 - 15.55	14.68 ± 0.77	20.78 - 22.4	21.84 ± 0.79
49	FFTF	7.18 -8.7	7.75 ± 0.68	9.82 - 11.02	10.4 ± 0.53
50	WTF	2.81 - 3.22	3.03 ± 0.16	5.72 - 6.99	6.39 ± 0.58
51	WFF	5.11 - 5.29	5.17 ± 0.07	6.88 - 7.79	7.24 ± 0.45
52	T1	7.08 - 7.65	7.32 ± 0.23	11.67 -12.67	12.06 ± 0.37
53	T2	10.61 - 11.63	11.11 ± 0.43	15.44 - 17.27	16.48 ± 0.66
54	T3	15,74 - 16.72	16.13 ± 0.04	22.71 - 24.54	23.82 ± 0.72
55	T4	21.94 - 23.49	22.38 ± 0.66	31.1 - 32.56	32.17 ± 0.32
56	T5	17.71 - 19.73	18.68 ± 0.75	25.82 - 27.49	26.79 ± 0.65

5.3. Breeding Behavior:

1. Breeding Behavior of *Rhacophorus maximus:* During the study period, it was observed that *Rhacophorus maximus* is a seasonal breeder and its breeding activity coincides with the onset of monsoon season i.e. February and continued up to the month of April. The study was conducted both in study sites I and II. The atmospheric temperature recorded ranged between 26ºC to 38ºC, water temperature between 24ºC to 28ºC, pH between 5.54 - 8.22 during the investigation period in both the study sites.

Courtship and advertisement calls

During the study period, adult male frogs were observed to be the first to emerge from their hiding places during the evening at around 4 p.m. and make advertisement calls. It was also observed that multiple males aggregate and produce advertisement call while hiding behind the grasses, some damp places near the breeding site, or while floating on the water surface. Advertisement calls were audible to the human ear from a distance of about 10 –15 m. The calling sound was usually heard during the evening and continued till early in the morning. It was observed that the call was remarkably high in the evening after rainfall. Calls were also heard during in during the peak breeding period.

Advertisement calls were emmited in series with variable call intervals. The call consisted of a single note (Fig. 5.3.1) emmited at an interval of 0.8 - 0.9s. The notes lasted 0.4 s and were composed a series of 2.0 pulses. The amplitude of the note increased quickly in its second third and decrease until the end. The frequency spectra had a dominant band at 1464.258 Hz.

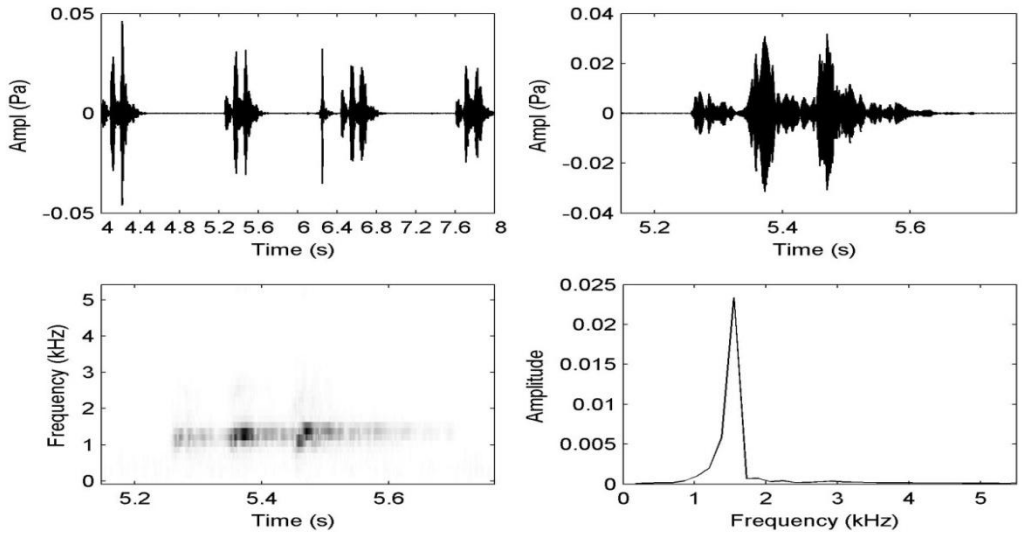

Fig. 5.3.1: Oscillogram, sonogram and frequency spectrum of an advertisement call of *Rhacophorus maximus*

Mating and Spawning

In this study, vocalization of unsuccessful male was noticed from evening to the next morning. The female frog, attracted by the calls, emerged and responded towards the breeding ground. After entering the breeding ground, the male frog then emerged from its hiding place and encircled the female. It then suddenly grasped the female resulting in axillary amplexus (Fig. 5.3.2). Amplexus was observed to take place both during the day and night-time. In the present

study, combating behavior of males was also observed, where one male frog tried to dislodge another amplecting pair. Pairs in amplexus can be seen in water submerging together usually in the corner of the pool. Amplecting was axillary and a single pair was found in the study site I, whereas two to three pairs were also encountered in the study site II at a time. Amplexus was observed to last for one to several hours before the nest construction took place and the female deposited its eggs in a large creamy white foam nest. The nests were observed to be constructed on diverse substrata. Some of the foam nests constructed were attached to the grasses and stones on the side of the pond and also floating on the water surface. Both male and female frogs left the nests after construction and no parental care was observed. Fresh laid eggs were collected from the breeding sites and were monitored in the laboratory.

Fig. 5.3.2: Axillary amplecting in *Rhacophorus maximus*

2. Breeding behavior of *Polypedates teraiensis* (Dubois, 1987):

During the study period, it was observed that the breeding activity of *Polypedates teraiensis* starts from March where the onset of monsoon had already started in Mizoram. From March to August the frogs came out to mate in the pools. The atmospheric temperature recorded ranges between 26ºC to 38ºC, water temperature between 24ºC to 28ºC, pH between 5.54 - 8.22 during the investigation period in both the study sites I and II.

Courtship and advertisement calls:

During the study period, adult male frogs were observed to be the first to emerge from their hiding places during the evening at around 3.30 p.m. and make advertisement calls while perching on the grasses, twigs and branches of trees near the breeding sites. Advertisement calls were audible to the human ear from a distance of about 20–30 m. The calling sound was usually heard during the evening and continued till early in the morning. It was observed that the call was remarkably high in the evening and night time after rainfall.

Advertisement calls were emitted in series with variable call intervals (Fig. 5.3.3). The notes lasted 0.5 s and were composed a single pulse. The notes interval ranges from 0.9 - 10 s. The frequency spectra have a dominant band at 1981.055 Hz and the band width ranges from 359.5705 to 1570.255 Hz.

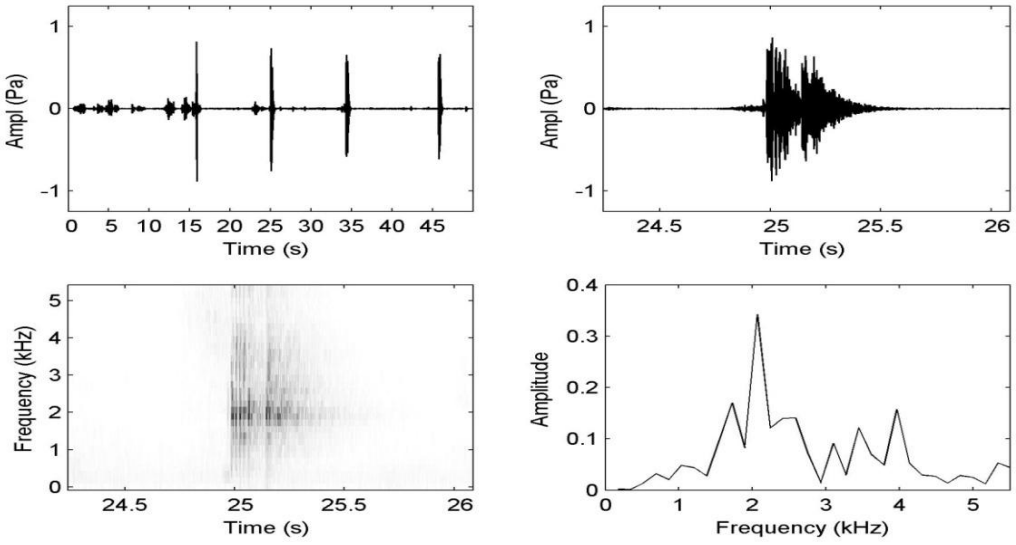

Fig. 5.3.3: Oscillogram, sonogram and frequency spectrum of an advertisement call of *Polypedates teraiensis*

Mating and Spawning

In this study, vocalization of unsuccessful male was noticed from evening to the next morning. During the mating period, male first enter the breeding ground and starts advertisement calls. In respond to male's vocalization, female entered the breeding pool from the surrounding forest. Female approach male slowly, there is no visual cue. Male then suddenly grasped the female resulting in axillary amplexus. Amplexus was observed to take place both during the day and night-time. In the present study, combating behavior of males was observed, where one male frog tried to dislodge another amplecting pair. Amplecting was axillary (Fig.4.3.4) and sometimes two to three pairs were found in the study site I, whereas one to two pairs were also

encountered in the study sites II. Amplexus was observed to last for 40-90 minutes before the oviposition took place. The female deposited its eggs in a sphere like structure with about 10 - 15 cm in a diameter which are whitish to light brown in color. Generally, their oviposition sites are on the substrata which is in touch with water or on the surface of water. As the tadpoles of *Rhacophorus maximus* which were already hatch out feed on the foam nest and embryos of *Polypedates teraiensis*, so *Polypedates teraiensis* shifted their oviposition site about 0.1m to the wall of the water tank in the breeding sites or leaves and twigs of the plant high above the water bodies. After deposition of eggs and no parental care was observed. Fresh laid eggs were collected from the breeding sites and were monitored in the laboratory.

Fig. 5.3.4: Axillary amplecting in *Polypedates teraiensis*

5.4. Development and Metamorphosis:

1. Development and Metamorphosis of *Rhacophorus maximus*:

During the course of this study the developmental stages of the 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 also reared and monitored in the laboratory. The stages in the entire developmental series were selected on the basis of external morphological characteristics as described by Gosner (1960), and altogether 46 different developmental stages

were recorded for each species. For each stages, five numbers were used for morphometric measurement.

A brief account of various stages of development and metamorphosis of *Rhacophorus maximus* was given in the following sections.

Stage 1 –Fertilized egg: The freshly layed egg was spherical in shape with the animal hemisphere pigmented dark brown. It is surrounded by a thin, transparent, vitelline membrane. It measures about 2.15 ± 0.07 mm (Fig. 5.4.1).

Stage 2 – Gray crescent: A faintly gray crescent is noticeable on the animal pole of the egg. The vegetal hemisphere is more prominent than earlier. It measures about 2.31 ± 0.08 mm. This stage is observed within 0:25 hr (Fig. 5.4.2).

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 2.46 ± 0.05mm. The first cleavage was completed in 1:20 hr after fertilization (Fig. 5.4.3).

Stage 7 – Thirty two cell stage: 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 6:25 hr to complete and measures 2.53 ± 0.03mm (Fig. 5.4.4).

Stage 10 – Dorsal lip: The extension of the diving 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 8:00 hr. The egg measured 2.60 ± 0.03mm (Fig. 5.4.5).

Stage 11 – Yolk plug: 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 9:30 hr and the gastrula measured 2.69 ± 0.02 mm (Fig. 5.4.6).

Stage 13 – Neural plate: The embryo was 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 2.72 ± 0.05 mm. This stage was observed at 12:00 hr (Fig. 5.4.7).

Stage 14 – Neural fold: The embryo was 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 15:45 hr and the embryo measured 2.81 ± 0.06mm (Fig. 5.4.8).

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 18:05 hr and measured 2.89 ± 0.03 mm (Fig. 5.4.9).

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 2.98 ± 0.01mm. This stage was observed at 20:35 hr (Fig. 5.4.10).

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. Completion of this stage was observed after 1 day. Embryo measured 3.36 ± 0.05 mm (Fig. 5.4.11).

Stage 21 – Cornea transparent: After 3 days the cornea was transparent. Tail became straight and elongated Oral suckers and nasal pits became prominent. The embryo measured 11.57 ± 0.25 mm. The larva hatched out from its eggs at this stage (Fig. 5.4.12).

Stage 22 – Tail fins Circulation: Tail fins become transparent and circulated, and the epidermis was pigmented. The head and trunk are distinctly demarcated. The oral suckers are prominent and nipple shaped. This stage is observed after 5 days. It measured 12.76 ± 0.39 mm (Fig. 5.4.13).

Stage 23 – Labia and teeth differentiate: After 6 days the operculum developed, and gills length shortened. The labia and teeth can be differentiated. The larva hatched out from the gelatin covers and it measured 13.45 ± 0.19 mm (Fig. 5.4.14).

Stage 24 – Operculum closes on right side: It was observed that right operculum fold closed after 8 days and left external gill shortened. The body length is 13.58 ± 0.22 mm (Fig. 5.4.15).

Stage 25 – Spiracle form on left side: After 8 days, left operculum fold was closed and completion of spiracle takes place on the left side. The external gills have completely regressed and the tadpole measured 13.65 ± 0.20 mm (Fig. 5.4.16).

Stage 26 – Hind limb bud < ½ its diameter: The tadpole measured 15.26 ± 0.53mm. Hind limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 14 days. Coiled intestine became visible. Rows of teeth were observed in both the upper and lower labium posteriorly (Fig. 5.4.17).

Stage 27– Length of limb bud ≥ ½ its diameter: The length of the limb bud length equaled to half of its diameter is observed after 16 days. The total length was 20.42 ± 0.18 mm. Oral structure does not change (Fig. 5.4.18).

Stage 28 – Length of limb bud ≥ its diameter: Length of limb bud equaled to its diameter on the 18th day. The total length was 25.96 ± 0.61 mm (Fig. 5.4.19).

Stage 29 – Length of limb bud ≥ 1 ½ its diameter: After about 19 days, the limb bud becomes more elongated and it was about one and half of its width. The tadpole length was 27.70 ± 0.90 mm (Fig. 5.4.20).

Stage 30 – Length of limb bud = twice its diameter: The limb bud further increased in length and becomes conical. The tadpole length was 28.85 ± 1.17 mm. The bud was still without any pigment, and there was no change in the oral structure. It was observed after 22 days (Fig. 5.4.21).

Stage 31 – Foot paddle: After 24 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 30.43 ± 1.57 mm from snout to tail tip (Fig. 5.4.22).

Stage 32 – First interdigital indentation: After 26 days, margin of the foot paddle slightly indented that seperates the prominence of the 4^{th} and 5^{th} toes (Fig. 5.4.23).

Stage 33 – Second interdigital indentation: After 27 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 tadpole length was 33.40 ± 0.35 mm (Fig. 5.4.24).

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 28 days, the tadpole attained 33.42 ± 0.64 mm (Fig. 5.4.25).

Stage 35 – Fourth interdigital indentation: After 31 days the total length of tadpole was about 41.78 ± 8.19 mm. The margin of the foot paddle was also indented between toes 1st and 2nd and all five toes were separated from each other (Fig. 5.4.26).

Stage 36 – Separation of 3-5 toes: After 33 days, the total length of tadpole was 40.58 ± 0.01 mm. The 1st and 2nd toes were still joined, while the 3rd, 4th and 5th toes were separated (Fig. 5.4.27).

Stage 37 – Toes separation completed: All toes were completely separated and webbed after 35 days. The total length of tadpole was about 40.91 ± 0.05 mm (Fig. 5.4.28).

Stage 38 – Appearance of Metatarsal tubercles: Metatarsal tubercles showed its appearance at the base of 1st toe after 37 days, and the total length of tadpole was 43.79 ± 0.99 about mm (Fig. 5.4.29).

Stage 39 – Appearance of Subarticular patches: Subarticular tubercles first appeared as parches after 40 days, and the total length of tadpole was about 50.34 ± 0.59 mm (Fig. 5.4.30).

Stage 40 – Vent tube present: After 42 days, toes were with fully developed subarticular patches and fully webbed. The total length of tadpole was about 48.49 ± 0.76 mm (Fig. 5.4.31).

Stage 41 – Vent tube gone: After 45 days, the more drastic changes of metamorphosis began. 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 41.72 ± 2.70 mm (Fig. 5.4.32).

Stage 42 – Emergence of fore limbs: On the 48th day, the total length of tadpole was about 39.68± 1.27 mm. Tadpole developed both fore limbs and hind limbs. Mouth started widening, and it was anterior to nostril. In this observation, right fore limb emerge first (Fig. 5.4.33).

Stage 43 – Tail Atrophies: Resorption of tail started to regress after 50 days and the length of tadpole was about 36.59 ± 0.46 mm. The lateral margin of mouth reached between nostril and eye. Shedding of teeth was completed (Fig. 5.4.34).

Stage 44 – Tail Greatly Reduced: The tail was greatly reduced. The total length of tadpole was about 22.13 ± 0.01 mm. There was further widening of mouth after 51 days (Fig. 5.4.35).

Stage 45 – Tail Stub: After 52 days, resorption of the tail was completed and only a stub remained, the tadpole measured 17.09 ± 0.39 mm in length. Tongue had fully developed (Fig. 5.4.36).

Stage 46 – Metamorphosis completed: After 53 days, the tail was completely reasorbed and the frog-let looks like a miniature adult and measured 16.18 ± 0.31 mm (Fig. 5.4.37).

Table 3: Age and size of developing *Rhacophorus maximus* embryos

(N= Total number of samples examined).

Sl. No.	Stage	Age	Size in mm (N = 5)
1.	Fertilization	0 hr	2.15 ± 0.07
2.	Gray Crescent	0:25 hr	2.31 ± 0.08
3.	2- cell	1:20 hr	2.46 ± 0.05
4.	32 - cell	6:25 hr	2.53 ± 0.03
5.	Dorsal Lip	8:00 hr	2.60 ± 0.03
6.	Yolk Plug	9:30 hr	2.69 ± 0.02
7.	Neutral Plate	12:00 hr	2.72 ± 0.05
8.	Neutral Fold	15:45 hr	2.81 ± 0.06
9.	Rotation	18:05 hr	2.89 ± 0.03
10.	Neural tube	20:35 hr	2.98 ± 0.01
11	Tail bud	1 day	3.36 ± 0.05
12.	Cornea Transparent	3 days	11.57 ± 0.25
13.	Tail Fin Circulation	5 days	12.76 ± 0.39
14.	Operculum present	6 days	13.45 ± 0.19
15.	Left Gill	8 days	13.58 ± 0.22
16.	Spiracles Forms	11 days	13.65 ± 0.20
17.	L < ½D	14days	15.26 ± 0.53
18.	L ≥ ½D	16 days	20.42 ± 0.18
19.	L ≥D	18 days	25.96 ± 0.61

20.	L ≥ 1½D	19 days	27.70 ± 0.90
21.	L ≥ 2D	22 days	28.85 ± 1.17
22.	Foot Paddle	24 days	30.43 ± 1.57
23.	Indentation 4-5	26 days	31.28 ± 0.68
24.	Indentation 3-4	27 days	33.40 ± 0.35
25.	Indentation 2-3	28 days	33.42 ± 0.64
26.	Indentation 1-2	31 days	41.78 ± 8.19
27.	Toes 3-5 Separated	33 days	40.58 ± 0.01
28.	All Toes Separated	35 days	40.91 ± 0.05
29.	Metatarsal tubercles	37 days	43.79 ± 0.99
30.	Sub - articular patches	40 days	50.34 ± 0.59
31.	Foot Tubercles	42 days	48.49 ± 0.76
32.	Fore Limbs Visible	45 days	41.72 ± 2.70
33.	Fore Limbs Emerge	48 days	39.68 ± 1.27
34.	Tail Atrophies	50 days	36.59 ± 0.46
35.	Tail Greatly Reduced	51 days	22.13 ± 0.01
36.	Tail Stub	52 days	17.09 ± 0.39
37.	Metamorphosis Complete	53 days	16.18 ± 0.31

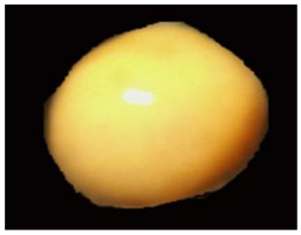

Fig. 5.4.1:Stage 1:Fertilization
0 hour

Fig. 5.4.2:Stage 2: Gray Crescent
0:25 hour

Fig. 5.4.3: Stage 3:
2- cell
1:20 hour

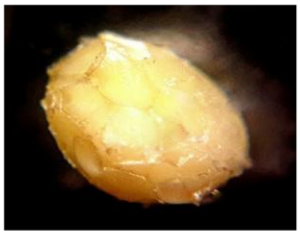

Fig. 5.4.4: Stage 7:
32- cell
6:25 hour

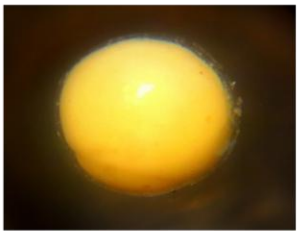

Fig. 5.4.5: Stage 10
Dorsal Lip
8:00 hour

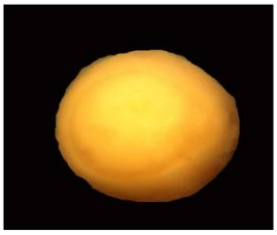

Fig. 5.4.6: Stage 11: Yolk Plug
9:30 hour

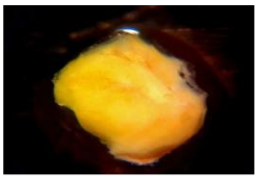

Fig. 5.4.7:Stage 13: Neural Plate
12:00 hour

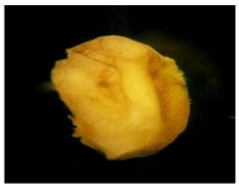

Fig. 5.4.8:Stage 14: Neural Folds
15:45 hour

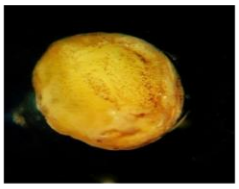

Fig. 5.4.9:Stage 15: Rotation
18:05 hour

Fig. 5.4.10:Stage 16: Neural Tube
20:35 hour

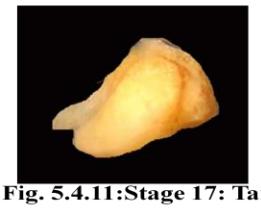

Fig. 5.4.11:Stage 17: Tail Bud
1 day

Fig. 5.4.12:Stage 21:Cornea Transparent
3 days

Fig. 5.4.13:Stage 22: Tail Fin Circulation
5 days

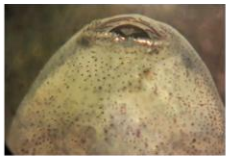

Fig. 5.4.14:Stage 23: Labia and teeth differentiate
6 days

Fig. 5.4.15:Stage 24: Operculum closes on Right
8 days

Fig. 5.4.16:Stage 25: Spiracles form on Left
11 days

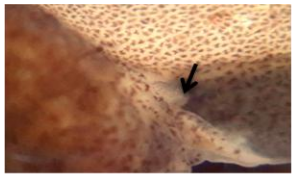

Fig. 5.4.17:Stage 26: L<1/2D
14 days

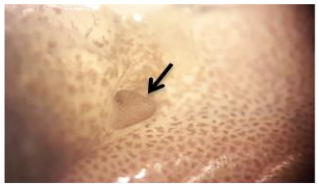

Fig. 5.4.18:Stage 27: L≥1/2D
16 days

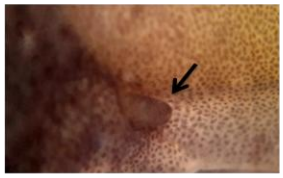

Fig. 5.4.19: Stage 28: L≥D
18 days

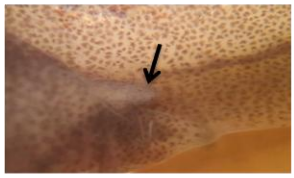

Fig. 5.4.20:Stage 29: L≥11/2D
19 days

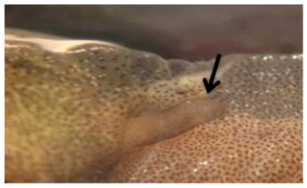

Fig. 5.4.21:Stage 30: L=2D
22 days

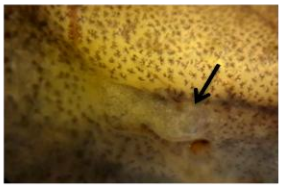

Fig. 5.4.22:Stage 31: Foot Paddle
24 days

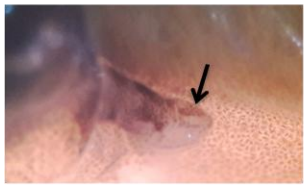

Fig. 5.4.23:Stage 32: Indentation 4-5
26 days

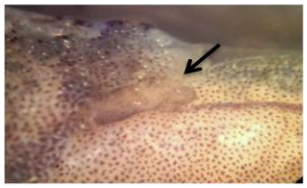

Fig. 5.4.24:Stage 33: Indentation 3-4
27 days

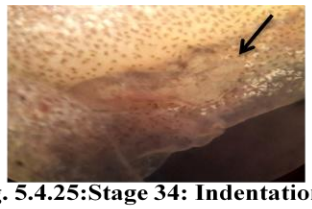

Fig. 5.4.25:Stage 34: Indentation 2-3
28 days

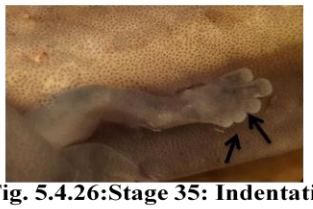

Fig. 5.4.26:Stage 35: Indentation 1-2
31 days

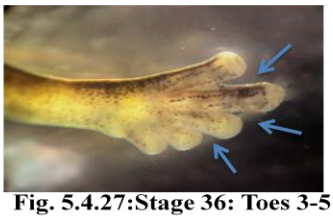

Fig. 5.4.27:Stage 36: Toes 3-5 Separated
33 days

Fig. 5.4.28:Stage 37: All Toes Separated
35 days

Fig. 5.4.29:Stage 38: Metatarsal Tubercles
37 days

Fig. 5.4.30:Stage 39: Sub- articular patches
40 days

Fig. 5.4.31:Stage 40: Vent tube present
42 days

Fig. 5.4.32:Stage 41: Fore-limb visible
45 days

Fig. 5.4.33:Stage 42:Fore-limb Emerge
48 days

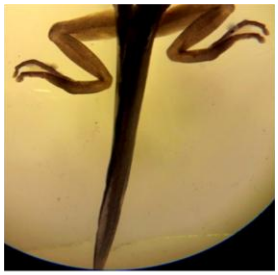

Fig. 5.4.34:Stage 43: Tail Atrophies
50 days

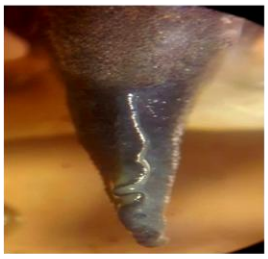

Fig. 5.4.35 (a): Stage 44:Tail Greatly Reduced
51 days

Fig. 5.4.35(b): Stage 44: Whole body in
dorsal view

Fig. 5.4.36:Stage 45: Tail stub
52 days

Fig. 5.4.37:Stage 46: Metamorphosis Complete
53 days

Fig. 5.4.1 - 37: Stages of development and metamorphosis of *Rhacophorus maximus*

Tadpoles of *Rhacophorus maximus* were also measured in different developmental stages using a dial caliper accurate to 0.02 mm. Five numbers (N=5) of tadpoles were measured in each different developmental stages (Table 4).

Table 4: Morphometeric measurements (in mm) of tadpoles of *Rhacophorus maximus*

	Operculum, oral disc and pigmentation	**Hind limb bud development**	**Toe differentiation and development**					**Metamorphic stage**
Parameters	**Stage 25 (N=5)**	**Stage 27 (N=5)**	**Stage 31 (N=5)**	**Stage 33 (N=5)**	**Stage 36 (N=5)**	**Stage 38 (N=5)**	**Stage 41 (N=5)**	**Stage 43 (N=5)**
TL	14.56 ± 0.24	21.45 ± 0.30	27.12 ± 0.63	31.92 ± 1.12	41.07 ± 1.73	43.31 ± 1.80	45.62 ± 0.81	35.94 ± 0.73
TAL	7.99 ± 0.35	13.77 ± 0.66	17.37 ± 0.35	21.02 ± 0.83	26.14 ± 1.39	25.87 ± 2.89	29.61 ± 0.28	21.05 ± 0.56
BL	4.18± 0.66	8.12 ± 0.50	10.03 ± 0.49	11.68 ± 1.07	15.27 ± 1.06	15.50 ± 1.63	17.55 ± 0.85	14.52 ± 0.92
BW	3.60 ± 0.12	5.58 ± 0.25	6.28 ± 0.43	6.44 ± 0.17	8.51 ± 0.20	8.26 ± 0.16	10.66 ± 0.65	7.37 ± 0.12
IOD	2.27± 0.05	2.39 ± 0.14	2.50 ± 0.16	2.97 ± 0.49	3.86 ± 0.33	3.30 ± 0.20	5.12 ± 0.90	3.84 ± 0.61
IND	1.69 ± 0.39	1.3 ± 0.14	1.28 ± 0.09	1.39 ± 0.05	2.88 ± 0.08	2.38 ± 0.31	2.39 ± 0.58	2.35 ± 0.66
SO	2.7 ± 0.08	2.79 ± 0.28	2.71 ± 0.29	3.68 ± 0.32	4.86 ± 0.83	4.22 ± 0.21	4.86 ± 0.65	3.33 ± 0.15
SN	1.31 ± 0.36	1.65 ± 0.40	1.51 ± 0.29	2.07 ± 0.01	3.34 ± 0.78	2.94 ± 0.56	2.96 ± 0.81	2.71 ± 0.25
MTH	3.45 ± 0.21	3.82 ± 0.67	5.59 ± 0.36	5.39 ± 0.23	7.78 ± 0.50	6.92 ± 0.67	7.73 ± 0.40	5.27 ± 0.62
TMW	2.51 ± 0.31	2.39 ± 0.60	2.81 ± 0.64	2.80 ± 0.14	3.83 ± 0.64	4.26 ± 0.11	5.45 ± 0.74	2.35 ± 0.02

2. Development and metamorphosis of *Polypedates teraiensis* :

During the study period, foam nests of *Polypedates teraiensis* were collected from the study sites and brought to the laboratory for rearing. The developmental stages of *Polypedates teraiensis* were recorded from the time of egg laying till the completion of metamorphosis from March to May during the study period in the laboratory at water temperature between 19°C and 27°C. The eggs of *Polypedates teraiensis* took 3-4 days for hatching after the collection. The eggs are non-pigmented. The life history (post hatching) was completed within 61 - 64 days.

A brief account of stages of development and metamorphosis of *Polypedates teraiensis* is given in the following.

Stage 1 –Fertilized egg: The freshly layed egg was spherical in shape with the animal hemisphere pigmented light brown and the yolky vegetal hemisphere white. It was surrounded by a thin, transparent, vitelline membrane (Fig. 5.4.38).

Stage 10 – Dorsal lip: The extension of the diving 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. The egg measured 1.52 ± 0.09 mm (Fig. 5.4.39).

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. The gastrula measured 1.62 ±0.08 mm (Fig. 5.4.40).

Stage 12 – Late gastrula: The small protruding blastopore gradually was reduced due to constriction of the blastoporal lips. The late gastrula measured 1.73 ± 0.11 mm (Fig. 5.4.41).

Stage 13 – Neural plate: The embryo was slightly elongated and the dorsal surface has flattened to form the neural plate, and the lateral ridges became slightly elevated forming neural folds. This stage is observed at 1 day 6 hours (Fig. 5.4.42).

Stage 14 – Neural fold: The embryo was 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 1 day 10 hours and the embryo measured 1.87 ± 0.05mm (Fig. 5.4.43).

Stage 15 – Rotation: The elevated neural folds growth towards each other and the neural groove became narrow. The embryo was now more elongated antero-posteriorly. It was completed at 1 day 15 hours and measured 2.05 ± 0.06 mm (Fig. 5.4.44).

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 2.49 ± 0.06 mm. This stage was observed at 1 day 18 hours (Fig. 5.4.45).

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. Completion of this stage was observed at 1 day 23 hours. Embryo measured 3.38 ± 0.36mm (Fig. 5.4.46).

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 2 hours. The embryo measured 4.49 ± 0.18 mm (Fig. 5.4.47).

Stage 19 – Heart beat: Heart beat was observed at 2 days 7 hours and the embryo measured 5.18± 0.32 mm (Fig. 5.4.48).

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 embryo measured 7.18 ± 0.49 mm and it was observed within 3 days 4 hours. Hatching of larvae were observed at this stage (Fig. 5.4.49).

Stage 21 – Cornea transparent: After the cornea was transparent. Tail became straight and elongated Oral suckers and nasal pits became prominent. The embryo measured 8.92 ± 0.39 mm (Fig. 5.4.50).

Stage 22 – Tail fins transparent: Tail fins become transparent, and the epidermis is pigmented. The head and trunk are distinctly demarcated. The oral suckers were prominent and nipple shaped. This stage was observed after 3 days 22 hours. It measured 10.28 ± 0.34 mm (Fig. 5.4.51).

Stage 23 – Operculum covers gill base: After 4 days the operculum developed, and gills length shortened. It was measured 11.36 ± 0.59 mm (Fig. 5.4.52).

Stage 24 – Operculum closes on right side: It was observed that right operculum fold closed after 8 days and left external gill shortened. The tadpole measured 15.62 ± 0.74 mm (Fig. 5.4.53).

Stage 25 – Spiracle form on left side: After 8 days, left operculum fold was closed and completion of spiracle takes place on the left side. The external gills have completely regressed and the tadpole measured 15.62 ± 0.74 mm (Fig. 5.4.54).

Stage 26 – Hind limb bud < ½ its diameter: The tadpole measured 19.42 ± 0.98 mm. Hind limb buds appeared at the junction of the trunk and tail on either side of the cloacal tail after 16 days. Coiled intestine became visible. Rows of teeth were observed in both the upper and lower labium posteriorly (Fig. 5.4.55).

Stage 27– Length of limb bud ≥ ½ its diameter: The length of the limb bud length equaled to half of its diameter is observed after 18 days. The total length was 21.67 ± 0.7 mm (Fig. 5.4.56).

Stage 28 – Length of limb bud ≥ its diameter: The total length was 23.55 ± 0.49 mm; Length of limb bud equaled to its diameter on the 20th 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. 5.4.57).

Stage 29 – Length of limb bud ≥ 1 ½ its diameter: After about 23 days, the limb bud becomes more elongated and it was about one and half of its width. The tadpole length was 25.89 ±0.3 mm (Fig. 5.4.58).

Stage 30 – Length of limb bud = twice its diameter: The limb bud further increased in length and becomes conical. The tadpole length was 27.05 ±0.14 mm. The bud was still without any pigment, and there was no change in the oral structure. It was observed after 26 days (Fig. 4.4.59).

Stage 31 – Foot paddle: After 28 days, the total length of the tadpole was 28.65 ± 0.39 mm from snout to tail tip (Fig. 5.4.60).

Stage 32 – First interdigital indentation: After 30 days the margin of the foot paddle became slightly indented on the dorsal side, which separates the prominences of the 4th and 5th toes (Fig. 5.4.61).

Stage 33 – Second interdigital indentation: After 32 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 30.75 ± 0.45 mm (Fig. 5.4.62).

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 34 days, the tadpole attained 31.94 ± 0.12 mm (Fig. 5.4.63).

Stage 35 – Fourth interdigital indentation: After 35 days the total length of tadpole was about 32.76 ± 0.39 mm. The margin of the foot paddle was also indented between toes 1st and 2nd and all five toes were separated from each other (Fig. 5.4.64).

Stage 36 – Separation of 5-4 and 4-3 toes: After 37 days, the total length of tadpole was about 33.9 ± 0.33 mm. The 1st and 2nd toes were still joined, while the 3rd, 4th and 5th toes were separated (Fig. 5.4.65).

Stage 37 – Toes separation completed: All toes were completely separated and webbed after 39 days. The total length of tadpole was about 35.1 ± 0.71mm (Fig. 5.4.66).

Stage 38 – Appearance of Metatarsal tubercles: Metatarsal tubercles showed its appearance at the base of 1st toe after 41 days, and the total length of tadpole was about 38.21 ± 0.58 mm (Fig. 5.4.67).

Stage 39 – Appearance of Subarticular patches: Subarticular tubercles first appeared as parches after 43 days, and the total length of tadpole was about 42.45 ± 1.22 mm (Fig. 5.4.68).

Stage 40 – Completion of foot tubercles: After 45 days, toes were with fully developed subarticular patches and fully webbed. The total length of tadpole was about mm; the body length was 44.2 ± 0.66 mm (Fig. 5.4.69).

Stage 41 – Vent tube gone: After 49 days, the more drastic changes of metamorphosis began. The cloacal tail piece was atrophied and the total of tadpole was about 42.29 ± 1.15 mm (Fig. 5.4.70).

Stage 42 – Emergence of fore limbs: On the 55 days, the total length of tadpole was about 38.63 ± 1.01 mm. Tadpole developed both fore limbs and hind limbs. Mouth started widening, and it was anterior to nostril. Right side of fore limb emerged first at this stage (Fig. 5.4.71).

Stage 43 – Mouth between nostril and eye: Resorption of tail started to regress after 57 days and the length of tadpole was about 26.11 ± 1.25 mm. The lateral margin of mouth reached between nostril and eye. Shedding of teeth was completed (Fig. 5.4.72).

Stage 44 – Mouth beneath eye: There was further widening of mouth after 58 days and the tail was greatly reduced and the total length of tadpole was about 18.18 ± 0.23 mm. Formation of the tongue took place (Fig. 5.4.73).

Stage 45 – Mouth posterior to eye: After 59 days, resorption of the tail was completed and only a stub remained and tadpole measured 16.8 ± 0.33 mm in length. And the mandible extended beyond the eye. Tongue had fully developed (Fig. 5.4.74).

Stage 46 – Metamorphosis completed: After 61-64 days, the tail was completely reasorbed and the frog-let looks like an adult and measured 15.55 ± 0.37 mm (Fig. 5.4.75).

Table 5: Age and size of developing *Polypedates teraiensis* embryos

(N= Total number of samples examined)

Sl. No	Stages	Gosner stage	Age	Size in mm (N=5)
1.	Fertilization	1	0 hr	1.34 ± 0.07
2.	Dorsal lip	10	15:30 hr	1.52 ± 0.09
3.	York Plug	11	20:20 hr	1.62 ± 0.08
4.	Late Gastrula	12	1 day 2 hr	1.73 ±0.11
5.	Neural Plate	13	1 day 6 hr	1.82 ± 0.07
6.	Neural Fold	14	1 day 10 hr	1.87 ± 0.05
7.	Rotation	15	1 day 15 hr	2.05 ± 0.06
8.	Neural Tube	16	1 day 18 hr	2.49 ± 0.06
9.	Tail Bud	17	1 day 23 hr	3.38 ± 0.36
10.	Muscular Response	18	2 days 2 hr	4.49 ± 0.18
11.	Heart Beat	19	2 days 7 hr	5.18 ± 0.32
12.	Tail Elongation	20	3 days 4 hr	7.18 ± 0.49
13.	Cornea Transparent	21	3 days 9 hr	8.92 ± 0.39
14.	Tail Fin Transparent	22	3 days 22 hr	10.28 ± 0.34
15.	Operculum Present	23	4 days	11.36 ± 0.59
16.	Left Gill	24	6 days	12.95 ± 0.72
17.	Spiracles Form	25	8 days	15.62 ± 0.74
18.	L < ½D	26	16 days	19.42 ± 0.98
19.	L ≥ ½D	27	18 days	21.67 ± 0.7
20.	L ≥D	28	20 days	23.55 ± 0.49
21.	L ≥ 1½D	29	23 days	25.89 ± 0.3
22.	L ≥ 2D	30	26 days	27.05 ± 0.14
23.	Foot Paddle	31	28 days	28.65 ± 0.39
24.	Indentation 4-5	32	30 days	29.88 ± 0.41
25.	Indentation 3-4	33	32 days	30.75 ± 0.45
26.	Indentation 2-3	34	34 days	31.94 ± 0.12
27.	Indentation 1-2	35	35 days	32.76 ± 0.39
28.	Toes 3-5 Separated	36	37 days	33.9 ±0.33
29.	All Toes Separated	37	39 days	35.1 ± 0.71
30.	Metatarsal Tubercles	38	41 days	38.21 ± 0.58
31.	Sub Articular Patches	39	43 days	41.45 ± 1.22
32.	Foot Tubercles	40	45 days	44.2 ± 0.66
33.	Fore Limbs Visible	41	49 days	42.29 ± 1.15
34.	Fore Limbs Emerge	42	55 days	38.63 ± 1.01

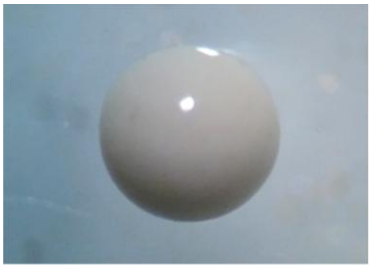

Fig. 5.4.38: Stage 1: Fertilization
0 hour

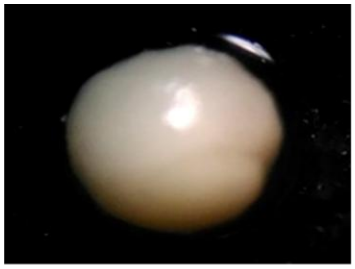

Fig. 5.4.39: Stage 10 Dorsal Lip
15:30 hours

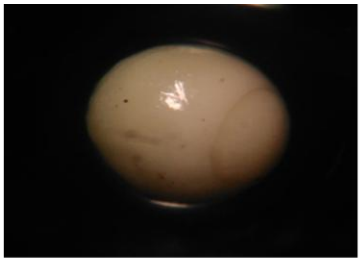

Fig. 5.4.40: Stage 11: Yolk Plug
20:20 hours

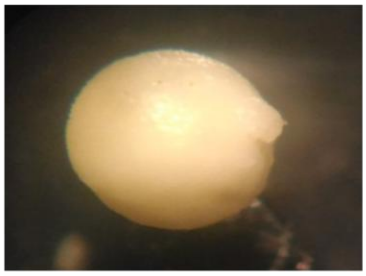

Fig. 5.4.41: Stage 12: Late Gastrula
1 day 2 hours

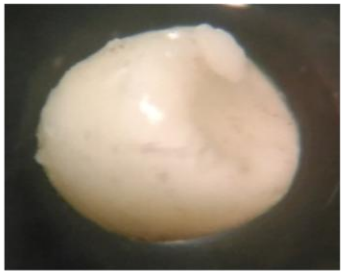

Fig. 5.4.42: Stage 13: Neural Plate
1 day 6 hours

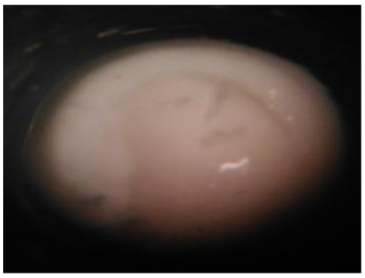

Fig. 5.4.43: Stage 16: Neural Tube
1 day 10 hours

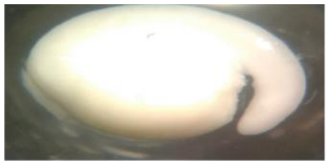

Fig. 5.4.44: Stage 17: Tail Bud
1 day 15 hours

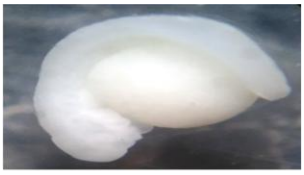

Fig. 5.4.45: Stage 18: Muscular Response
1 day 18 hours

Fig. 5.4.46: Stage 19: Heart Beats
1 day 23 hours

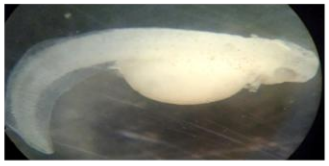

Fig. 5.4.47: Stage 20: Gill Circulation
2 days 2 hours

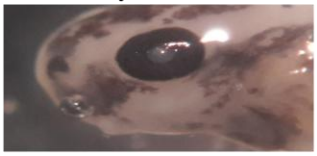

Fig. 5.4.48: Stage 21:Cornea Transparent
2 days 7 hour

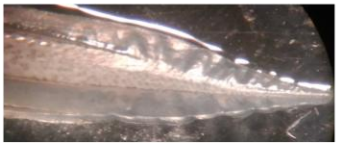

Fig. 5.4.49: Stage 22: Tail Fin Circulation
3 days 4 hours

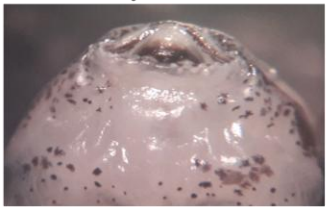

Fig. 5.4.50: Stage 23: Labia and teeth differentiate
3 days 9 hours

Fig. 5.4.51: Stage 24: Left Gill
3 days 22 hours

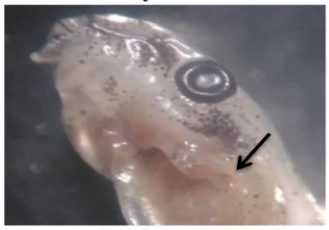

Fig. 5.4.52: Stage 25: Spiracles form on Left
4 days

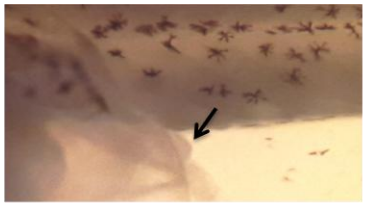

Fig. 5.4.53: Stage 26: L<1/2D
6 days

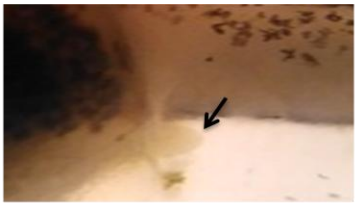

Fig. 5.4.54: Stage 27: L≥1/2D
8 days

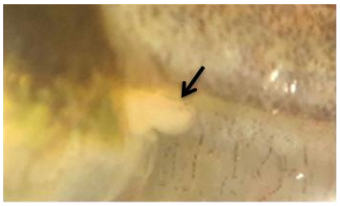

Fig. 5.4.55: Stage 28: L≥D
16 days

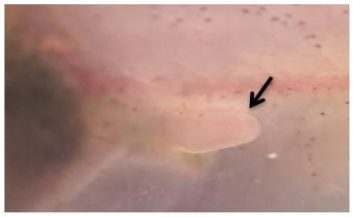

Fig. 5.4.56: Stage 29: L≥11/2D
18 days

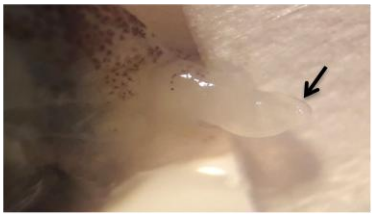

Fig. 5.4.57: Stage 30: L=2D
20 days

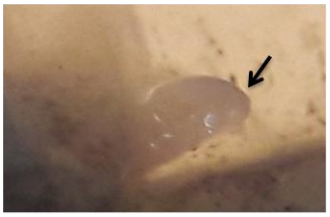

Fig. 5.4.58: Stage 31: Foot Paddle
23 days

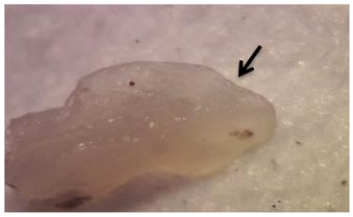

Fig. 5.4.59: Stage 32: Indentation 4-5
26 days

Fig. 5.4.60: Stage 33: Indentation 3-4
31 days

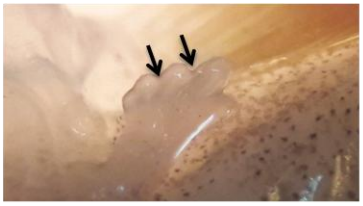

Fig. 5.4.61: Stage 34: Indentation 2-3
32 days

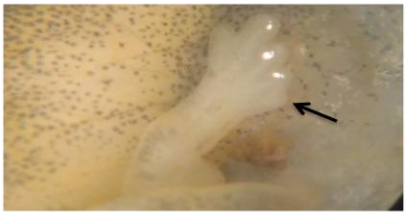

Fig. 5. 4.62:Stage 35: Indentation 1-2
34 days

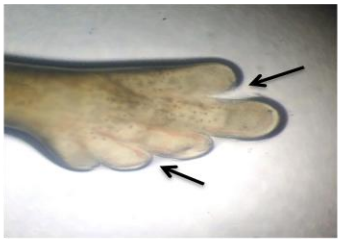

Fig. 5.4.63:Stage36: Toes 3-5 Separated
35 days

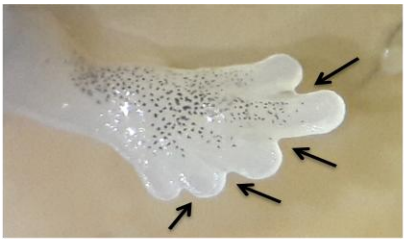

Fig. 5.4.64:Stage 37: All Toes Separated
37 days

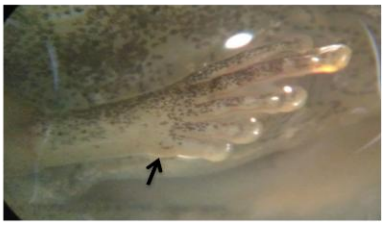

Fig. 5.4.65:Stage 38: Metatarsal Tubercles
39 days

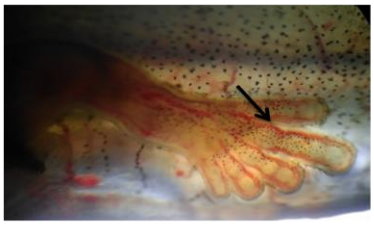

Fig. 5.4.66:Stage 39: Subarticular patches
41 days

Fig. 5.4.67:Stage 40: Foot Tubercles
43 days

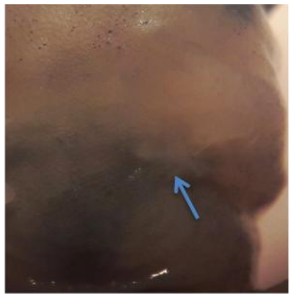

Fig. 5.4.68:Stage 41: Forelimb visible
45 days

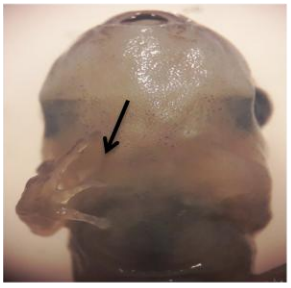

Fig. 5.4.69:Stage 42: Forelimb emerge
49 days

Fig. 5.4.70:Stage 43: Tail Atrophies
55 days

Fig. 5.4.71:Stage 44: Tail Greatly Reduced
58 days

Fig. 5.4.72:Stage 45: Tail Stub
59 days

Fig. 5.4.73:Stage 46: Metamorphosis Complete
61-64 days

Fig. 5.4.38 – 73: Stages of development and metamorphosis of *Polypedates teraiensis*

Measurement of tadpoles of *Polypedates teraienis* were also taken in different developmental stages. Five numbers (N=5) of tadpoles were measured in each different developmental stages (Table 6).

Table 6: Morphometric measurements (in mm) of tadpoles of *Polypedates teraiensis* in different developmental stages

	Operculum, oral disc and pigmentation	Hind limb bud development	Toe differentiation and Development				Metamorphic stage	
Parameters	**Stage 25 (N=5)**	**Stage 27 (N=5)**	**Stage 31 (N=5)**	**Stage 33 (N=5)**	**Stage 36 (N=5)**	**Stage 38 (N=5)**	**Stage 42 (N=5)**	**Stage 44 (N=5)**
TL	15.88 ± 0.55	21.45 ± 0.30	27.07 ± 0.71	31.66 ± 1.29	34.02 ± 3.07	40.33± 1.76	39.34 ± 1.28	23.73 ± 0.30
TAL	9.52 ± 0.35	13.77 ± 0.66	17.33 ± 0.46	20.80 ± 1.12	22.56 ± 1.59	22.63 ± 2.43	27.09 ± 1.69	7.28 ± 0.65
BL	4.92 ± 0.63	8.12 ± 0.50	10.01 ± 0.57	11.85 ± 1.25	12.88 ± 1.28	13.68 ± 1.88	12.48 ± 0.99	16.56 ± 1.02
BW	3.60 ± 0.12	5.58 ± 0.25	6.23 ± 0.49	6.52 ± 0.16	8.51 ± 0.26	7.72 ± 0.84	6.20 ± 0.15	6.22 ± 0.48
IOD	2.27 ± 0.05	2.39 ± 0.14	2.54 ± 0.16	3.08 ± 0.64	3.82 ± 0.42	2.88 ± 0.62	4.64 ± 0.28	3.96 ± 0.87
IND	1.69 ± 0.39	1.3 ± 0.14	1.3 ± 0.17	1.39 ± 0.07	2.92 ± 0.08	2.2 ± 0.33	2.55 ± 0.31	2.0 ± 0.83
SO	2.7 ± 0.08	2.79 ± 0.28	2.66 ± 0.32	3.79 ± 0.32	4.69 ± 1.10	4.05 ± 0.34	3.16 ± 0.08	3.15 ± 0.72
SN	1.31 ± 0.36	1.65 ± 0.40	1.53 ± 0.34	2.08 ± 0.01	3.36 ± 0.91	2.60 ± 0.65	1.36 ± 0.26	1.81 ± 0.04
MTH	3.45 ± 0.21	3.82 ± 0.67	5.60 ± 0.42	5.43 ± 0.31	8.01 ± 0.54	6.57 ± 0.80	5.59 ± 1.30	3.36 ± 0.62
TMW	2.56 ± 0.10	2.39 ± 0.60	2.83 ± 0.72	2.82 ± 0.16	4.02 ± 0.83	3.99 ± 0.45	2.82 ± 0.58	3.29 ± 0.63

5.5. Food and feeding behavior in relation to oral structures.

Qualitative analysis of gut contents of the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis* revealed that the larvae started feeding from stage 25 onwards. The list of all the food items in the gut of tadpoles and its percent abundance and percent frequency occurrence are shown in table 7 and 9.

1. Larval foods of *Rhacophorus maximus* Günther, 1858:

Stage 25: From this stage it was observed that tadpoles start feeding. The gut contents of the tadpoles contain phytoplankton consisting of *Spirogyra* and *Polycystis* (Chlorophyceae), *Navicula*, *Cyclotella* and *Tabellaria* (Bacillariophyceae), *Phacus* and *Euglena* (Euglenoidea) and non-phytoplankton like *Notholca* (Zooplankton). Among phytoplanktons, one unidentified food item was observed.

Stage 26-30: At these stages, the gut content of the tadpoles is similar to that of Stage 25 except in additions of phytoplankton like *Netrium* (Mesoteniaceae) and non-phytoplankton like *Lecane* (Zooplankton).

Stage 31-41: Analysis of the gut content of the tadpoles at these stages reveals that the tadpoles also feed on phytoplanktons like *Mougeotia*, *Microspora* and *Ulothrix* (Chlorophyceae) apart from the food items on the previous stages.

Stage 42-46: At these stages tadpoles still feed on phytoplanktons and Zooplanktons but few in numbers. The tadpoles at these stages start to lose its feeding larval structures. From Stage 46 onwards, the froglet starts feeding on carnivorous diet.

Table 7: Qualitative and quantitative list of food items found in the gut of tadpoles of *Rhacophorus maximus* and percent relative abundance and percent frequency of occurrence

Stage 25: Operculum, Oral discs and pigmentation

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Spirogyra	15	80	65.21
Polycystis	8	80	34.78
Bacillariophyceae			

Navicula	35	80	62.5
Cyclotella	10	40	17.85
Tabellaria	11	20	19.64
Euglenophyceae			
Phacus	2	20	66.67
Euglena	1	20	33.34
Zooplanktons			
Notholca	6	100	100
Unidentified	13	20	100

Stage 26 – 30: Hind Limb Bud Development

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Polycystis	555	100	98.40
Spirogyra	9	100	1.59
Bacillariophyceae			
Navicula	27	100	81.81
Cyclotella	3	40	9.09
Tabellaria	3	20	9.09
Euglenophyceae			
Phacus	594	100	99.16
Euglena	5	20	0.83
Mesotaeniaceae			
Netrium	70	40	100
Zooplanktons			
Notholca	32	80	96.96
Lecane	1	20	3.03
Unidentified	3	20	100

Stage 31- 41: Toe Differentiation and Development

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Polycystis	1751	100	96.68
Spirogyra	11	37.5	0.60
Mougeotia	32	12.5	1.76
Microspora	12	25	0.66
Ulothrix	5	12.5	0.27
Bacillariophyceae			
Navicula	137	100	84.56
Cyclotella	8	25	4.93
Tabellaria	17	25	10.49
Euglenophyceae			
Phacus	139	87.5	77.65
Euglena	40	37.5	22.34
Mesoteniaceae			
Netrium	30	62.5	100
Unidentified	88	62.5	100

Stage 42-46: Metamorphic stage

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Polycystis	67	80	78.82
Protococcus	5	20	5.88
Spirogyra	4	40	4.70
Mougeotia	9	20	10.58
Bacillariophyceae			
Navicula	28	60	82.35
Diatoma	6	40	17.64

Euglenophyceae			
Phacus	1	20	100
Mesotenaiceae			
Netrium	5	20	100
Zooplanktons			
Notholca	2	20	100

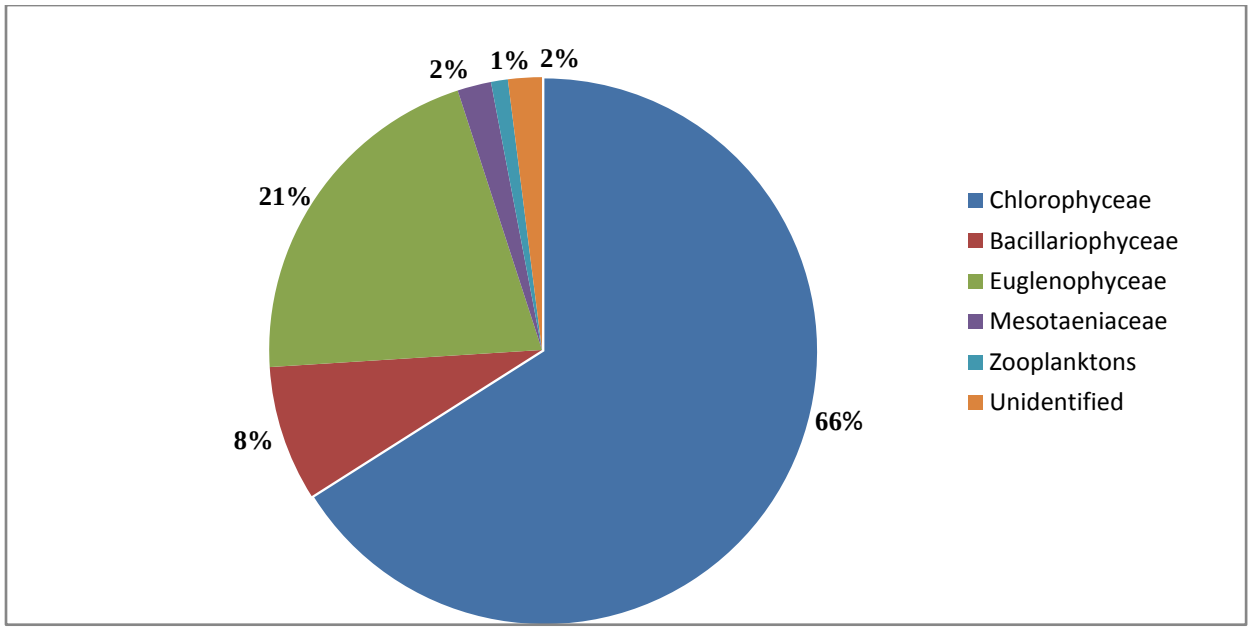

Fig. 5.5.1: Diet composition (%) of tadpoles of *Rhacophorus maximus*

In *Rhacophorus maximus*, Chlorophyceae (66%) is the most frequent among the food items found in the gut of the tadpoles followed by Euglenophyceae (21%). Bacillariophyceae (8%), Meaoteineacea (2%), unindentified (2%) organisms and Zooplanktons (1%). Zooplanktons and Mesoteniaceae were comparatively poor than the other. Among Chlorophyceae, *Polycystis* species were most frequent and abundant followed by *Spirogyra* species.

Table 8: Estimates of Berger – Parker Diversity Index (1/d = reciprocal form), Shannon-Wiener Diversity Index (H') and Evenness (E) in the gut of *Rhacophorus maximus*

Gosner Stage	1/d	H'	E
25	0.028	1.968	0.85
26-30	0.02	1.224	0.49
31-41	0.006	0.497	0.20
42-46	0.134	1.24	0.56

An estimates of food diversity based on the reciprocal form of the Berger-Diversity Index (1/d) and Shannon-Wiener Diversity Index (H') reveals that the greatest degree of dominance in food items occurs at stage 42-46 and the highest species diversity found at stage 25. Species evenness (E) was constrained between 0 - 1.0 which shows that all the species are not equally abundant.

Oral structures of the tadpoles of *Rhacophorus maximus:* Oral morphology of tadpoles of *Rhacophorus maximus* show that as follows:

Stage 25: At Stage 25, tooth rows A - 1, A - 2, A - 3 and P - 1, P - 2 and P - 3 are formed where A - 2 was interrupted medially and P-1 was slightly interrupted in making LTRF: 3(2 - 3)/3(1) (Fig. 5.5.2).

Stage 26 - 29: At these stage, the mouth is more prominent, the upper labium has now four rows of labial teeth and three rows on the lower labium which makes LTRF: 4(2 - 4)/3(1) (Fig. 5.5.3).

Stage 30 - 41: At these stages, the mouth is widened. Tooth rows at the upper labium A - 1, A - 2, A - 3, A - 4, A - 5 and on the lower labium P - 1, P - 2 and P-3 are complete making LTRF: 5(2 - 5)/3(1) (Fig. 5.5.4).

Stage 42-26: From stage 42 onwards, the mouth parts degenerated and gradually transformed into adult mouth. After attaining stage 46, the froglet feeds on a carnivorous diet (Fig. 5.5.5).

Fig. 5.5.2: Stage 25, LTRF: 3(2 - 3)/3(1)

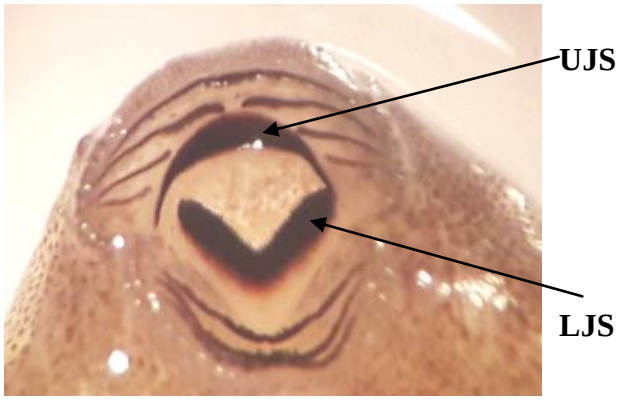

Fig. 5.5.3: Stage 26 - 29, LTRF: 4(2 - 4)/3(1)

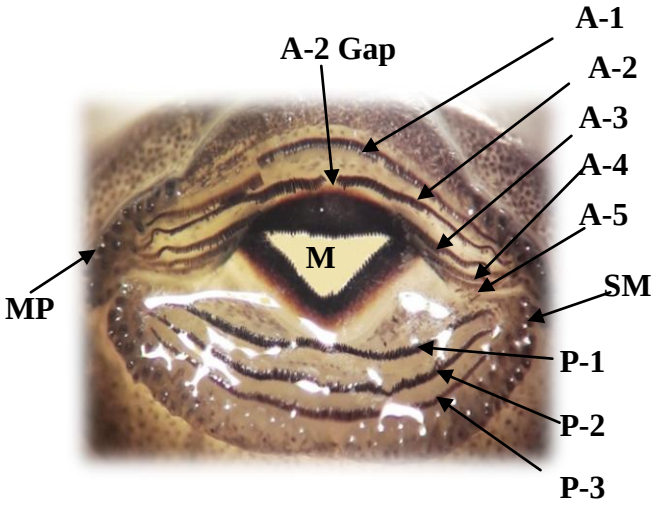

Fig. 5.5.4: Stage 30 - 41, LTRF: 5(2 - 5)/3(1)

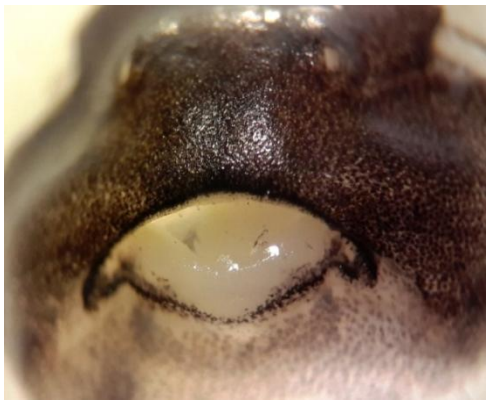

Fig. 5.5.5: Stage 42 - 46, Shedding of teeth

Fig. 5.5.2 - 5: Oral apparatus of *Rhacophorus maximus* under stereoscopic binocular microscope

Abbreviations: A - 1, A - 2, A - 3, A - 4 and A - 5 – first, second, third, fourth and fifth anterior tooth rows; P - 1, P - 2, P - 3 and P - 4 – first, second, third and fourth posterior tooth rows; E- emargination of oral disc; A - 2 Gap – medial gap in second anterior tooth row; UJS – upper jaw sheath; LSJ – lower jaw sheaths; MP- marginal papillae; SM- submarginal papillae; M– Mouth; OD – Oral Disc.

2. Larval foods of *Polypedates teraiensis* (Dubois, 1987):

Stage 25: It was observed that the tadpoles start feeding from Stage 25 onwards. The gut contents of tadpoles contain phytoplankton like *Polycystis* and *Spirogyra* (Chlorophyceae), *Phacus* (Euglenophyceae), and non-phytoplanktons like *Notholca* and *Lecane* (Zooplanktons) and Unidentified organisms.

Stage 26 - 30: At these stages, the gut content of the tadpoles is similar to that of Stage 25 except in additions of phytoplankton like *Navicula*, *Cyclotella* and *Tabellaria* (Bacillariophyceae) and *Euglena* (Euglenophyceae).

Stage 31 – 41: At these stages food items like *Mougeotia* (Chlorophyceae), *Genicularia* (Desmidiaceae) was observed apart from the food items occurs on the previous stages.

Stage 42 – 46: At these stages, the tadpoles start to lose its feeding structures and gradually transformed into adult mouth. The gut contents of the tadpoles content is similar to stage 26 - 30 but few in numbers. From Stage 46 onwards, the froglet starts feeding on carnivorous diet.

Table 9: Qualitative and quantitative list of food items found in the gut of tadpoles of *Polypedates teraiensis* and percent relative abundance and percent frequency of occurrence

Stage 25: Operculum, Oral discs and pigmentation

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Polycystis	75	100	76.53
Spirogyra	23	80	23.46
Euglenophyceae			
Phacus	14	80	100
Zooplanktons			
Notholca	2	40	66.67
Lecane	1	20	33.34
Unidentified	19	60	100

Stage 26 – 30: Hind Limb Bud Development

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Polycystis	619	100	98.09
Spirogyra	12	20	1.90
Bacillariophyceae			
Navicula	37	80	15.35
Cyclotella	40	40	16.59
Tabellaria	164	80	68.04
Euglenophyceae			
Phacus	152	80	92.12
Euglena	12	80	7.31
Zooplanktons			
Notholca	5	80	31.25
Lecane	11	60	68.75
Unidentified	50	80	72.46
Other Unidentified	19	80	27.53

Stage 31- 41: Toe Differentiation and Development

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Polycystis	846	87.5	93.37
Mougeotia	60	37.5	6.63
Bacillariophyceae			
Navicula	546	100	61.56
Cyclotella	129	100	14.54
Tabellaria	212	62.5	23.91
Euglenophyceae			
Euglena	80	100	44.45

Phacus	100	100	55.56
Desmidiacea			
Genicularia	8	25	100
Unidentified	254	100	96.55
Other Unidentified	9	37.5	3.42

Stage 42 - 46: Metamorphic stage

Food items	No. of organisms	Percent Frequency of occurrence	Percent Abundance
Chlorophyceae			
Polycystis	409	100	99.75
Mougeotia	1	33.4	0.24
Bacillariophyceae			
Navicula	115	100	45.81
Cyclotella	56	100	22.31
Tabellaria	80	100	31.87
Euglenophyceae			
Euglena	3	33.4	4.0
Phacus	72	100	96.0
Zooplanktons			
Notholca	3	66.7	75
Lecane	1	33.4	25
Unidentified	206	100	100

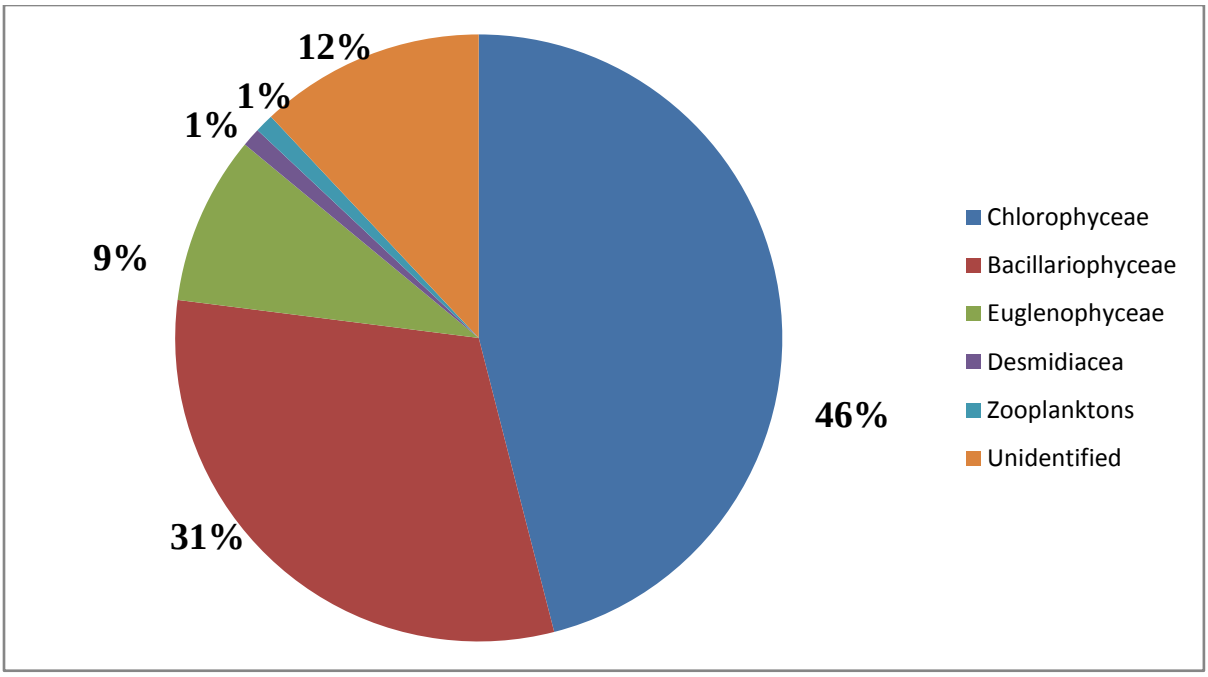

Fig. 5.5.6: Diet composition (%) of tadpoles of *Polypedates teraiensis*

Chlorophyceae were most frequent among the food items found in the gut of tadpoles of *Polypedates teraiensis* which is followed by Bacillariophyceae (31%), Euglenophyceae (9%) and Unidentified organisms (12%). Zooplanktons (1%) and Desmidiaceae (1%) were poor compared to other. In Chlorophyceae, the most frequently occurring species was *Polycystis* species followed by *Mougeotia* species.

Table 10: Estimates of Berger – Parker Diversity Index (1/d = reciprocal form), Shannon-Wiener Diversity Index (H') and Evenness (E) in the gut of *Polypedates teraiensis*

Gosner Stage	1/d	H'	E
25	0.09	1.43	0.73
26 - 30	0.01	1.49	0.60
31 - 41	0.01	2.78	1.08
42 - 46	0.02	1.56	0.75

Estimates of food diversity based on the reciprocal form of the Berger-Diversity Index (1/d) and Shannon-Wiener Diversity Index (H') reveals that the greatest degree of dominance in food items occurs at stage 25 and the highest species diversity found at stage 31 - 41. Species Evenness (E) constrained between 0.60 - 1.08 reveals that the species are not equally abundant.

Oral structures of the tadpoles of *Polypedates teraiensis:* Oral morphology of tadpoles of *Polypedates teraiensis* was studied under a stereoscopic binocular microscope (CETI 9554.1200).

Stage 25: At stage 25, the upper labium has one continuous and two discontinuous rows and the lower labium has one discontinuous and two continuous rows making LTRF: 3(2 - 3)/3(1) (Fig. 5.5.7).

Stage 26 - 30: At these stages, the mouth is more prominent, the upper labium has now four rows of labial teeth and three rows on the lower labium which makes LTRF: 4(2 - 4)/3(1) (Fig. 5.5.8).

Stage 31 - 41: At these stages, oral disc is more prominent than the previous stage and the LTRF was maintained (Fig. 5.5.8).

Stage 42 - 46: At stage 42 - 46, the mouth widens and modified into adult type, which finally metamorphosed into a froglet at stage 46 and starts feeding on a carnivorous diet (Fig. 5.5.9 - 10).

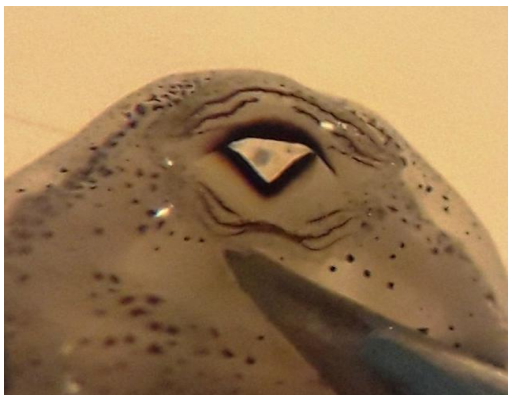

Fig. 5.5.7: Stage25, LTRF: 3(2 - 3)/3(1)

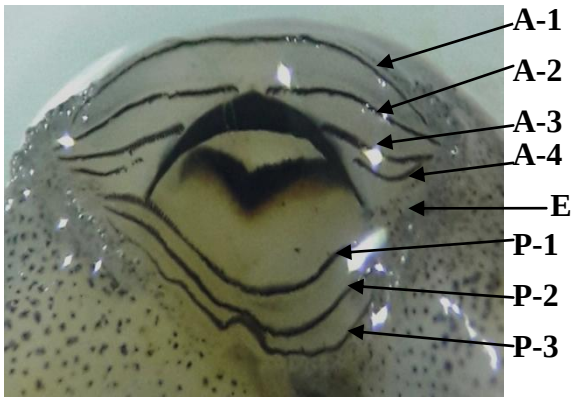

Fig. 5.5.8: Stage 26 - 41, LTRF: 4(2 - 4)/3(1)

Fig. 5.5.9: Stage 42: Mouth parts start to degenerate

Fig. 5.5.10: Stage 43 - 46: Mouth transformed into adult type

Fig. 5.5.7 - 10: **Oral apparatus of *Polypedates teraiensis* under stereoscopic binocular microscope**

Abbreviations: A - 1, A - 2, A - 3, A - 4 and A - 5 – first, second, third, fourth and fifth anterior tooth rows; P - 1, P - 2, P - 3 and P - 4 – first, second, third and fourth posterior tooth rows; E- emargination of oral disc; A - 2 Gap – medial gap in second anterior tooth row; UJS – upper jaw sheath; LSJ – lower jaw sheaths; MP- marginal papillae; SM- submarginal papillae; M– Mouth; OD – Oral Disc.

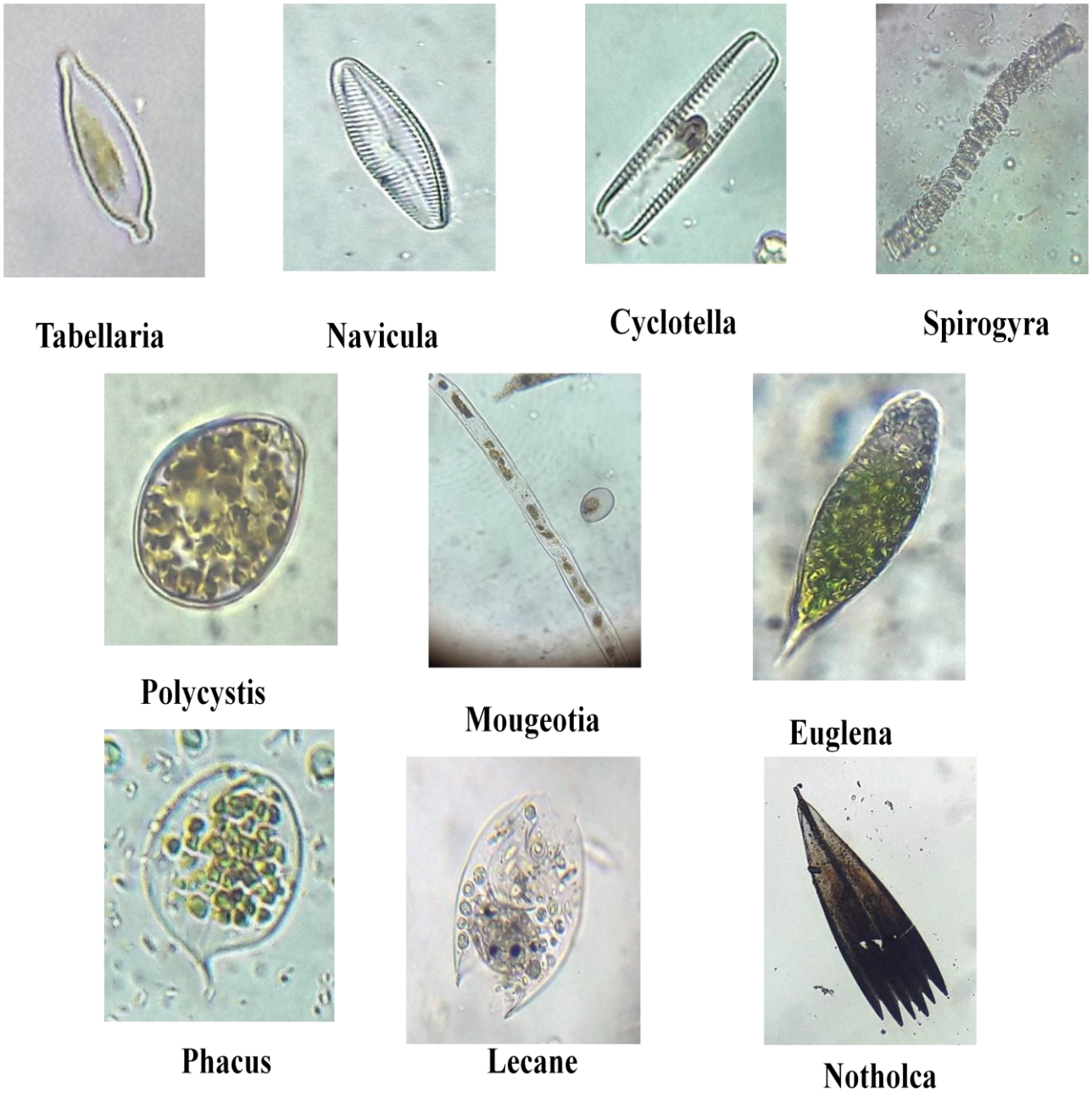

Fig. 5.5.11: Food items in the gut of tadpoles

Length of intestines:

1. Length of intestines of *Rhacophorus maximus*: In *Rhacophorus maximus*, the length of intestines of the tadpoles start increasing from stage 25, and there is a significant positive correlation between the Gosner stages from stage 25 to stage 40, where $p<0.01$ at the 0.01 level (2-tailed). From stage 41 - 46, the length of the tadpoles greatly reduced, showing significant positive correlation between Gosner stages with that of the total length and gut length where $p = 0.016$ at 0.05 level (2-tailed) as shown in the table 12 - 13. Measurement of the length of intestine of tadpoles at different developmental stages is shown in the table 11.

Table 11: Length of intestine of larval / tadpoles of *Rhacophorus maximus*

Gosner Stages	Total Length		Gut Length	
	Range (mm)	Mean ± SE	Range (mm)	Mean ± SE
Stage 25 (Operculum, Oral discs and pigmentation)	14.25 - 14.83	14.56 ± 0.24	20.6 - 20.31	20.13 ± 0.11
Stage 26 - 30 (Hind Limb Bud Development)	21.75 - 26.85	20.1 ± 2.08	34.96 - 70.63	59.79 ± 14.38
Stage 31 - 41 (Toe Differentiation and Development)	27.12 - 48.84	34.97 ± 7.64	70.63 - 180.72	106.27 ±38.70
Stage 42 - 46 (Metamorphic Stages)	15.38 - 42.12	26.54 ± 11.79	9.71 - 26.22	18.93 ± 7.27

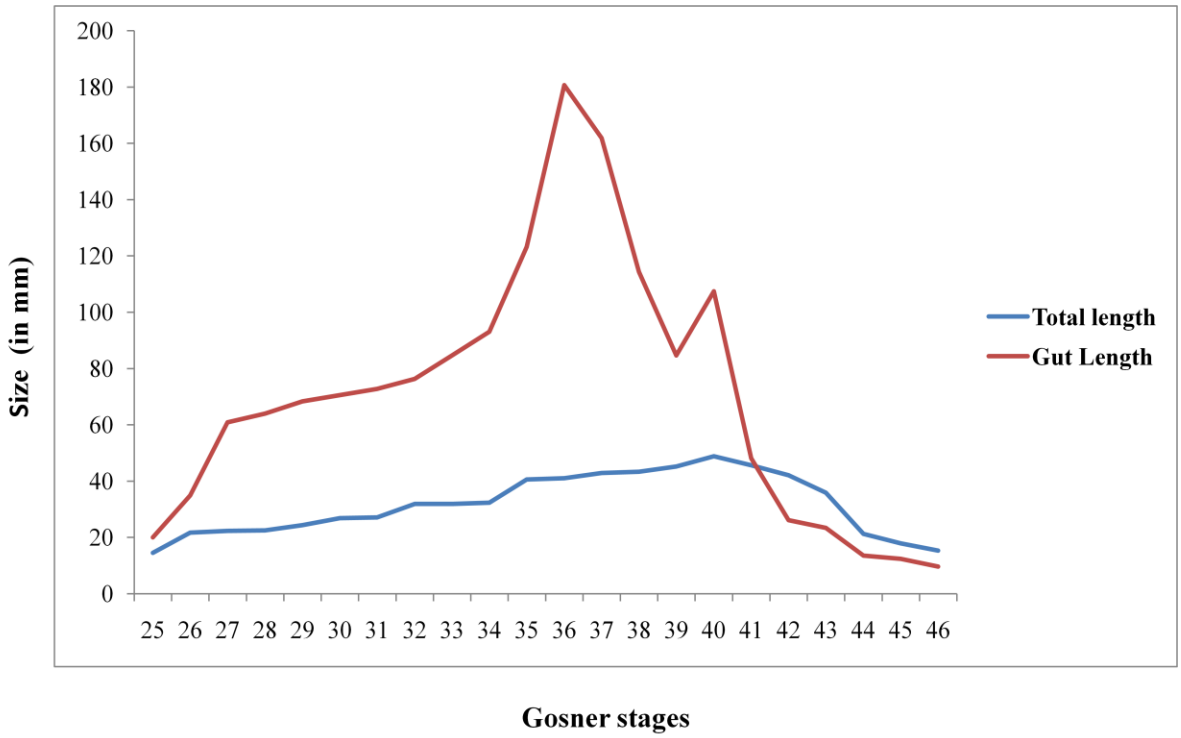

Fig. 5.5.12: Graph showing the changes of the total length and the gut length of tadpoles of *Rhacophorus maximus*

Table 12: Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Rhacophorus maximus*

Correlations				
		Stage	Total Length	Gut Length
Pearson Correlation	Stage	1	.985**	.762**
	Total Length	.985**	1	
	Gut Length	.762**	.792**	.792**
Sig.(2-tailed)	Stage		.000	.001
	Total Length	.000		.000
	Gut Length	.001	.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 13: Correlations between developmental stages from stage 41 to stage 46 along with total lengths and gut lengths of *Rhacophorus maximus*

Correlations				
		Stage	Total Length	Gut Length
Pearson Correlation	Stage	1	-.971**	-.912**
	Total Length	-.971**	1	.894*
	Gut Length	-.912**	.894*	
Sig.(2-tailed)	Stage		.001`	.011
	Total Length	.001		.016
	Gut Length	.011	.016	
N	Stage	6	6	6
	Total Length	6	6	6
	Gut Length	6	6	6
****. Correlation is significant at the 0.01 level (2-tailed).**				
***. Correlation is significant at the 0.05 level (2-tailed).**				

2. Length of intestines of *Polypedates teraiensis*: The length of tadpole intestine of *Polypedates teraiensis* were increased from stage 25 to stage 40.There was a significant positive correlation between the Gosner stages from stage 25 to stage 40, where $p<0.01$ at the 0.01 level (2-tailed). From stage 41-46, the length of the tadpoles greatly reduced, showing a significant positive correlation between developmental stages from stage 41-46, where p□0.01 at 0.01 level (2-tailed) as shown in the table 15 -16. The length of intestine of tadpoles at different developmental stages were measured as shown in the table 14.

Table 14: Length of intestine of larval/ tadpoles of *Polypedates teraiensis*

Gosner Stages	Total Length		Gut Length	
	Range (mm)	Mean ± SE	Range (mm)	Mean ± SE
Stage 25 (Operculum, Oral discs and pigmentation)	15.44 - 16.69	15.88 ± 0.55	16.02 - 16.31	15.97 ± 0.34
Stage 26 - 30 (Hind Limb Bud Development)	16.48 - 27.46	23.2 ± 4.56	17.35 - 77.76	38.95 ± 15.56
Stage 31 - 41 (Toe Differentiation and Development)	28.05 - 44.63	36.47 ± 5.06	33.54 - 124.94	87.60 ± 29.48
Stage 42 - 46 (Metamorphic Stages)	18.01 - 38.5	27.2 ± 8.71	8.24 - 28.15	18.26 ± 9.17

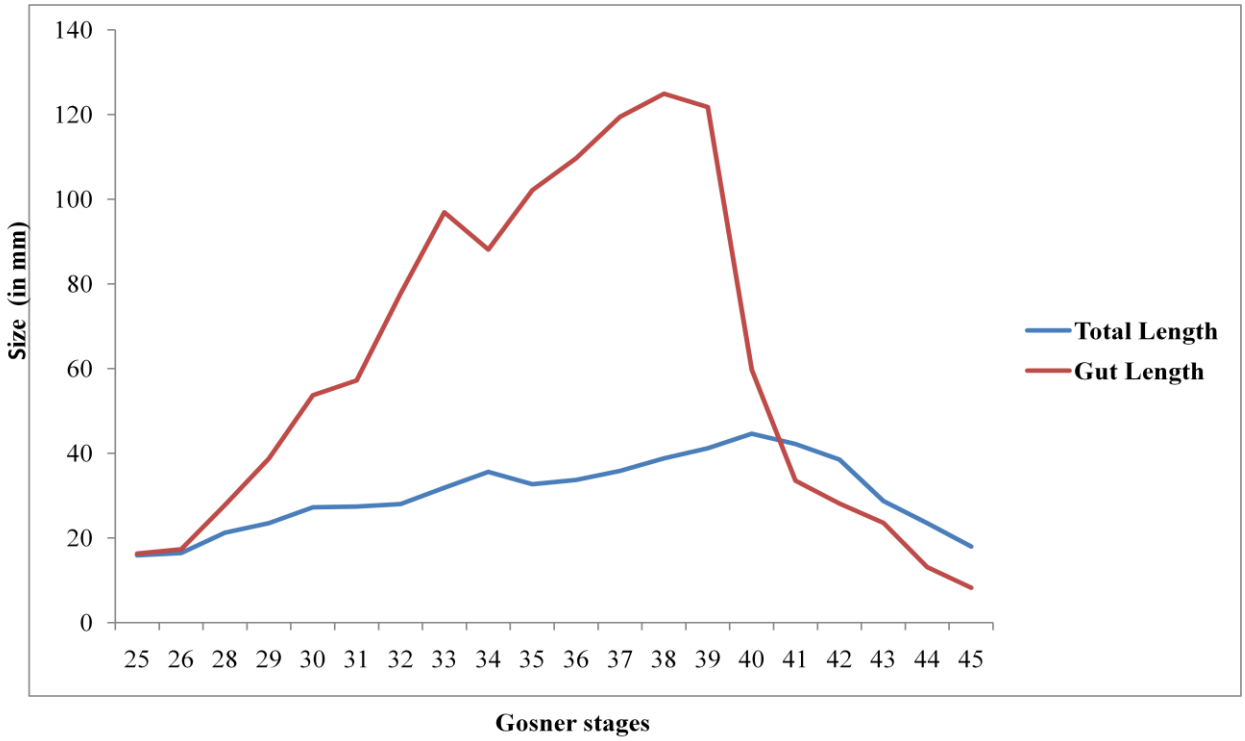

Fig. 5.5.13: Graph showing the changes of the total length and the gut length of tadpoles of *Polypedates teraiensis*

Table 15: Correlations between developmental stages from stage 25 to stage 40 along with total lengths and gut lengths of *Polypedates teraiensis*

Correlations				
		Stage	Total Length	Gut Length
Pearson Correlation	Stage	1	.977**	.847**
	Total Length	.977**	1	.801**
	Gut Length	.847**	.801**	1
Sig.(2-tailed)	Stage	.000	.000	.000
	Total Length	.000		.000
	Gut Length	.000	.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 16: Correlations between developmental stages from stage 41 to stage 46 along with total lengths and gut lengths of *Polpedates teraiensis*

Correlations				
		Stage	Total Length	Gut Length
Pearson Correlation	Stage	1	-.992**	.-990**
	Total Length	-.992**	1	.978*
	Gut Length	-.990**	.978*	1
Sig.(2-tailed)	Stage		.001	.001
	Total Length	.001		.004
	Gut Length	.001	.004	
N	Stage	5	5	5
	Total Length	5	5	5
	Gut Length	5	5	5
****. Correlation is significant at the 0.01 level (2-tailed).**				

CHAPTER 6

DISCUSSION AND CONCLUSION

The present study provides informations about the breeding behavior, food and feeding behavior and development of two sympatric rhacophorids, *Rhacophorus maximus* and *Polypedates teraiensis*. To understand their breeding behavior, survey was conducted from August, 2016 to July, 2017. Two study sites, an Artificial Pond located near Lianchhiari road, with a GPS location of N 23°44'15.7": E 92°40'02.5" at an elevation of 824 m asl and an old water storage tank located near Lengteng Boys Hostel, with a GPS location of N 23°44'18.0": E 92°39'43.2" at an elevation of 775 m asl were selected inside Mizoram University, Tanhril, Mizoram. The two study sites were found to be excellent breeding grounds and served as good habitat for the development and metamorphosis of this two sympatric species. Both *Rhacophorus maximus* and *Polypedates teraiensis* inhabit the same breeding sites. *Rhacophorus maximus* emerge first to the breeding ground followed by *Polypedates teraiensis*. The study sites were also utilized by other anurans like *Fejervarya cf limnocharis* (Dicroglossidae), *Duttaphrynus melanostictus* (Bufonidae), *Microhyla ornata* and *Kaloula pulchra* (Microhylidae).

It was observed that *Rhacophorus maximus* was an early and explosive breeder, as breeding activity and movements of frogs to the temporary rain fed pools and ponds for spawning are initiated with the first shower i.e., late February – March. The breeding activity is more or less similar with that of March to April with Khongwir *et. al.*, (2016) at Cherrapunjee and Mawsynram in Meghalaya. Advertisement call of *Rhacophorus maximus* was strongly pulsed, short, consisting of 2 pulses, and have frequency spectra with a dominant band of 1464.258 Hz and the band width ranges from 367.1037 to 692.2985 Hz. While the calls of another breeding species of *Rhacophorus* like *Rhacophorus belalongensis* were strongly pulsed consisting of only 1-4 pulses, and have high frequencies with energy maxima between 3500 and 6000 Hz (Dehling and Grafe, 2008). The water temperature for breeding and completion of life cycle in *Rhacophorus maximus* ranged between 21ºC to 30ºC, pH between 5.14- 8.75. The observation of the present study agreed with Khongwir *et. al*., (2016) where the species breeds after rainfall which leave standing water for the deposition of spawn. The breeding activity and movements of frogs to the temporary rain fed pools and ponds for spawning are initiated with an increase in temperature. In *Polypedates teraiensis*, the breeding activity was observed from March to August which is more or less similar with Chakravarty *et. al.*, (2011) who reported that *Polypedates teraiensis* begins to breed early in the year usually in the month of March. Tamuly and Dey (2014) observed the breeding activity of *Polypedates teraiensis* between the month of

April to August. The atmospheric temperature recorded for breeding and completion life cycle of *Polypedates teraiensis* ranges between 26ºC to 38ºC which is more or less similar with 26 ºC to 32 ºC by Chakravarty *et. al.,* (2011), and the water temperature ranges from 24ºC to 28ºC, pH between 5.54 - 8.22 during the investigation period in both the study sites. The advertisement call of *Polypedates teraiensis* was short consisting of a single pulse, and have frequency spectra with a dominant band of 1981.005 Hz and the band width ranges from 359.5705 to 1570.255 Hz which are more or less similar with that of Gogoi and Sengupta (2017) where calls consists of a single note of 1 to 6 pulses with a fundamental frequency ranges from 990.5 Hz to 1205.9 Hz. From the present investigation, it was confirmed that the breeding season of *Rhacophorus maximus* and *Polypedates teraiensis* coincided with early rainy season in Mizoram. It was suggested that the water temperature recorded might be the ambient temperature for each species. pH range was suggested as the optimal pH for the development of the species. From the data of advertisement call analysis, it was found that anuran calls are species-specific.

Distinct sexual dimorphism was observed in both the species where females are larger than males. Snout Vent Length (SVL) of amplecting females of *Rhacophorus maximus* ranges from 66.48 - 76.90 mm and from 67.89 - 75.22 mm in *Polypedates teraiensis*, whereas the SVL of males ranges from 47.88 - 70.14 mm in *Rhacophorus maximus* and from 52.93 - 56.45 mm in *Polypedates teraiensis*.

Amplexus was axillary where the male grasps the female at the axilla in both the species. Both the two rhacophorid frog deposit eggs in foam nest. The foam nests are thought to protect the eggs and embryos from desiccation, predators and thermal damage (Duellman and Trueb, 1994). Oviposition site is on the water surface of the breeding ground. The embryo of *Rhacophorus maximus* hatch out first and they exhibited carnivorous behavior, feeding on the foam nest and embryos of *Polypedates teraiensis. Polypedates teraiensis* then change their oviposition site about 0.1m on the wall of the water tank and later shifted to the leaves and trunks of plants high above the water. We suggested that shifting of oviosition site from a distance of water bodies might be for the avoidance of the predatory behavior of *Rhacophorus maximus* tadpoles. In both the species, no parental care was observed that is, after the construction of the nest, the male and female frogs leave the nest and

The duration for the development and completion of life cycle in *Rhacophorus maximus* was 52 - 53 days whereas within 61 - 64 days in *Polypedates teraiensis*. Other members of

Rhacophorid family such as *Rhacophorus malabaricus* completes metamorphosis in 68 days (Sekar, 1990), and *Rhacophorus arboreus* in 44 days (Iwasawa and Kawasaki, 1979). *Polypedates teraiensis* from Assam in 58 days (Chakravarty *et. al.*, 2011) however Tamuly and Dey (2014) reported that the life history of *P. teraiensis* was completed within 42 days. It was suggested that the difference in the duration of embryonic and larval period may be depending on the species.

During the study period, it was observed that the tadpoles from the breeding sites develop faster than the tadpoles that are reared in the laboratory and the size of the tadpoles were also larger than the tadpoles rearing from the laboratory. The size of the eggs of *Rhacophorus maximus* measures 2.15±0.07 mm (N=5) while eggs of *Polypedates teraiensis* measures 1.34±0.07mm (N=5), which are smaller than eggs of *Rhacophorus lateralis* measuring 2.8±0.5mm from Kalpetta, Wayanad District, Kerala. (Biju *et. al.,* 2009) and *Rhacophorus omeimontis* from South West China that measures 3.1 ± 0.1 mm (Liao and Lu, 2010). The embryo of *Rhacophorus maximus* start hatching at Gosner stage 21(11.57 ± 0.25 mm) and at stage 20 (7.18 ±0.49 mm) in *Polypedates teraiensis* which is similar with Chakravarty *et. al.,* (2011).The hind limb bud appeared at the junction of the trunk and tail on either side of the cloacal at stage 26 in both the species. In *Rhacophorus maximus,* the total body length of the tadpoles was maximum (50.34 ± 0.59 mm) at stage 39 and in *Polypedates teraiensis,* it was highest (44.2 ±0.6 mm) at stage 40. After this stage onwards, the body length, especially tail length decrease gradually and metamorphosis began. The right side of the forelimb emerged first followed by the left side of the limb at stage 42. At stage 44, tail was greatly reduced and metamorphosis was completed at stage 46.

The present study observed that tadpoles start feeding from stage 25 onwards. During the study period, herbivorous behavior was observed as the tadpoles feed on plants and algae, and cannibalism was also found among the two species. Cannibalism remains present throughout the animal kingdom and occurs most frequently under conditions of high density and food limitations (Crump, 1983). Orton (1953) formalized the concept of ecomorphological diversity of the anuran tadpoles, a scheme was again revised by Mc Diarmid and Altig (1999). The larvae or tadpoles of both the species, *Rhacophorus maximus* and *Polypedates teraiensis* can be put

under Orton's type 1.The tadpoles soon stop feeding at stage 42 and from stage 46 onwards the froglets feed on carnivorous diet. The gut contents of both the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis* were more or less similar and it also shows that they shared common in feeding habitats. Analysis of the intestinal contents of the tadpoles revealed that *Polycystis* belonging to the genus Chlorophyceae were the most dominant food items in the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis.* The stomach contents of both adults *Rhacophorus maximus* and *Polypedates teraiensis* were more or less similar. It was observed that the adult frogs consumed termites, beetles, dipterans, and small orthopterans, and pieces of leaves and twigs were also found in both the species.

Observation of the oral structures of the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis* reveals that the oral discs are positioned anteroventrally and slightly emarginated, and was composed of anterior (upper) labium and posterior (lower) labium. Marginal papillae and sub marginal papillae are present on the edge of the oral disc. The jaw sheath of *Rhacophorus maximus* was dark brown and serrated with a lower jaw sheath curved and with long appendices which agrees with Wildenhues *et. al.,* (2010). Whereas serrated jaw sheath with a lower jaw sheath large arch with weak median convexity of *Polypedates teraiensis* were observed, which is similar with Devi *et. al.,* (2016).The upper jaw sheaths were found to be V-shaped and the mouth is present in between in both species. The LTRF of *Rhacophorus maximus* was 3(2 - 3)/3(1) at stage 25, 4(2 - 4)/3(1) at stage 26 - 29 and 5(2 - 5)/3(1) from stage 30 onwards. Whereas the LTRF of *Polypedates teraiensis* was 3(2 - 3)/3(1) at stage 25 and from stage 26 onwards the tooth row formula was consistent at 4(2 - 4)/3(1) upto stage 41. Study shows that ontogeny i.e., changes in larval oral structure was observed in both the species. A gap in the tooth row is due to a physical break in the tooth ridge (Altig and Mc Diarmid, 1999). Altig and Johnston (1989) proposed that a tadpole with more tooth rows on the upper labium than the lower labium has a positive imbalance, and asummed that the differences in balance values reflected differences in feeding mode. Their oral structures starts to degenerate at stage 42 and gradually transformed into adult mouth and from stage 46 onwards, the froglet starts feeding on a carnivorous diet.

From the present investigation, it was observed among the two species that the length of intestines of the tadpoles start increasing from stage 25 to 40, and there is a significant positive correlation between the Gosner stages from stage 25 to stage 40, where $p<0.01$ at the 0.01 level (2-tailed). In *Rhacophorus maximus,* from stage 41 - 46, the length of the tadpoles were greatly

reduced, showing significant positive correlation between Gosner stages with that of the total length and gut length where p =0.016 at 0.05 level (2-tailed), whereas in *Polypedates teraiensis*, the length of the tadpoles also reduced which reveals that there is a positive correlation between developmental stages with that of the total length and the gut length where p□ 0.01 at 0.01 level (2-tailed). Our finding indicates that, in both *Rhacophorus maximus* and *Polypedates teraiensis*, as the tadpoles continue to grow, so does the intestine and as the tadpoles regresses from stage 41 onwards, intestine also begins to shorten to modified for carnivorous feeding behavior.

This study contributes on the information of breeding behavior, habit and habitat, development, food and feeding behavior of these two rhacophorids, *Rhacophorus maximus* and *Polypedates teraiensis* prevalent in Mizoram, can be useful for their conservation measures.

CHAPTER 7

SUMMARY

- The present study provides informations about the breeding behavior, food and feeding behavior and development of two sympatric rhacophorids, *Rhacophorus maximus* and *Polypedates teraiensis.*
- The two study sites were found to be excellent breeding grounds and served as good habitat for the development and metamorphosis of this two sympatric species.
- It was observed that *Rhacophorus maximus* is a seasonal breeder and its breeding activity coincides with the onset of monsoon season i.e., February to April.
- Advertisement call of *Rhacophorus maximus* was strongly pulsed, short, consisting of 2 pulses, and have frequency spectra with a dominant band of 1464.258 Hz and the band width ranges from 367.1037 to 692.2985 Hz.
- The water temperature for breeding and completion of life cycle in *Rhacophorus maximus* ranged between 21ºC to 30ºC, pH between 5.14- 8.75.
- The breeding activity of *Polypedates teraiensis* was observed from March to August.
- The advertisement call of *Polypedates teraiensis* was short consisting of a single pulse, and have frequency spectra with a dominant band of 1981.005 Hz and the band width ranges from 359.5705 to 1570.255 Hz.
- The atmospheric temperature recorded for breeding and completion life cycle of *Polypedates teraiensis* ranges between 26ºC to 38ºC and the water temperature ranges from 24ºC to 28ºC, pH between 5.54 - 8.22.
- Distinct sexual dimorphism was observed in both the species where females are larger than males. Snout Vent Length (SVL) of amplecting females of *Rhacophorus maximus* ranges from 66.48 - 76.90 mm and from 67.89 - 75.22 mm in *Polypedates teraiensis*, whereas the SVL of males ranges from 47.88 - 70.14 mm in *Rhacophorus maximus* and from 52.93 - 56.45 mm in *Polypedates teraiensis*.
- The duration for the development and completion of life cycle in *Rhacophorus maximus* was 52 - 53 days whereas within 61 - 64 days in *Polypedates teraiensis*.
- The size of the eggs of *Rhacophorus maximus* measures 2.15±0.07 mm (N=5) while eggs of *Polypedates teraiensis* measures 1.34±0.07 mm (N=5).
- The embryo of *Rhacophorus maximus* start hatching at Gosner stage 21(11.57 ± 0.25 mm) and at stage 20 (7.18 ±0.49 mm) in *Polypedates teraiensis.*

- In *Rhacophorus maximus*, the total body length of the tadpoles was maximum (50.34 ± 0.59 mm) at stage 39 and in *Polypedates teraiensis.*
- In both the species, the right side of the forelimb emerge first followed by the left side of the limb at stage 42. At stage 44, tail was greatly reduced and metamorphosis was completed at stage 46.

- Study observed that tadpoles start feeding from stage 25 onwards in both the species.
- During the study period, hervivorous behavior was observed as the tadpoles feed on plants and algae, and cannibalism was also found among the two species.
- The tadpoles soon stop feeding at stage 42 and from stage 46 onwards the froglets feed on carnivorous diet.
- Analysis of the intestinal contents of the tadpoles revealed that *Polycystis* belonging to the genus Chlorophyceae were the most dominant food items in the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis.*
- The stomach contents of both adults *Rhacophorus maximus* and *Polypedates teraiensis* were more or less similar. It was observed that the adult frogs consumed termites, beetles, dipterans, and small orthopterans, and pieces of leaves and twigs were also found in both the species.
- Observation of the oral structures of the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis* reveals that the oral discs are positioned anteroventrally and slightly emarginated, and was composed of anterior (upper) labium and posterior (lower) labium.
- The jaw sheath of *Rhacophorus maximus* was dark brown and serrated with a lower jaw sheath curved and with long appendices, whereas, serrated jaw sheath with a lower jaw sheath large arch with weak median convexity of *Polypedates teraiensis* were observed.
- The upper jaw sheaths were found to be V-shaped and the mouth is present in between in both species.
- LTRF of *Rhacophorus maximus* was 3(2 - 3)/3(1) at stage 25, 4(2 - 4)/3(1) at stage 26 - 29 and 5(2 - 5)/3(1) from stage 30 onwards, whereas, LTRF of *Polypedates teraiensis* was 3(2 - 3)/3(1) at stage 25 and from stage 26 onwards the tooth row formula was consistent at 4(2 - 4)/3(1) upto stage 41.
- Study shows that ontogeny i.e., changes in larval oral structure was observed in both the species.

- Their oral structures starts to degenerate at stage 42 and gradually transformed into adult mouth and from stage 46 onwards, the froglet starts feeding on a carnivorous diet.
- From the present investigation, it was observed among the two species that the length of intestines of the tadpoles start increasing from stage 25 to 40, and there is a significant positive correlation between the Gosner stages from stage 25 to stage 40, where $p<0.01$ at the 0.01 level (2-tailed).
- In *Rhacophorus maximus*, from stage 41 - 46, the length of the tadpoles were greatly reduced, showing significant positive correlation between Gosner stages with that of the total length and gut length where $p = 0.016$ at 0.05 level (2-tailed).
- In *Polypedates teraiensis*, the length of the tadpoles also reduced which reveals that there is a positive correlation between developmental stages with that of the total length and the gut length where p□ 0.01 at 0.01 level (2-tailed).
- In both *Rhacophorus maximus* and *Polypedates teraiensis*, it was observed that as the tadpoles continue to grow, so does the intestine and as the tadpoles regresses from stage 41 onwards, intestine also begins to shorten to modified for carnivorous feeding behavior.

CHAPTER 8

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APPENDICES

Published papers:

- Lalramdinfeli, M.H., and Lalremsanga, H.T. (2016). Observation on the breeding behaviour and analysis of the advertisement calls of *Rhacophorus maximus* Günther, 1858 (Anura: Rhacophoridae). *Science Vision,* **16 (4)**: 156-165.
- Lalramdinfeli, M.H., and Lalremsanga, H.T. (2017). Study on the emmbryonic development and metamorphosis of *Rhacophorus maximus* Günther, 1858 (Anura: Rhacophoridae). *Science and technology for shaping the future of Mizoram*, **12**: 59-68.

Papers Presentation at Seminars /Workshop / Conferences:

- Presented paper entitled **"Study on the embryonic development and metamorphosis of *Rhacophorus maximus* Günther, 1858 (Anura: Rhacophoridae)"** (14^{th} October, 2016) organized by Mizoram Science,Technology and Innovation Council (MISTIC) in association with Mizoram Science Society (MSS), Mizo Academy of Sciences (MAS), Geological Society of Mizoram (GSM), Science Teacher's Association of Mizoram (STAM), Mizoram Mathematics Society (MMS), and Biodiversity and Nature Conservation Network (BIOCONE).

Seminars / Training / Workshop / Conferences Participated:

- Workshop on **"National Level Workshop on Biostatistics and Bioinformatics"** (1^{th}–7^{th} September, 2016) organized by the Department of Biotechnology, Mizoram University.
- Workshop on **"Mechanisms of adaptation in the Temporal Environment"** (May 23, 2017) organized by the Department Zoology, Mizoram University, Aizawl, Mizoram.
- Outreach Program on **"Human Health and Biological Timing''** (May 22, 2017) organized by the Department of Zoology, Mizoram University, Aizawl, Mizoram.
- Workshop on **"Science Communication Workshop (SciComm 101)"** (6^{th} June, 2017) organized by the Department of Biotechnology, Mizoram University.
- Technical Session/ Exhibition in **Mizoram Science Congress** (13^{th}-14^{th} October, 2016) organized by MISTIC, MAS, STAM, MMS, GSM and BIOCONE.

CERTIFICATE

THIS IS TO CERTIFY THAT

~~Prof/Dr/Mr~~/Ms Mercy H. Lalramdinfeli

has presented (Oral/~~Poster~~) a paper entitled 'Study on breeding and developmental biology of **Rhacophorus maximus Gunther**, 1858 (Anura:Rhacophoridae).'
in Mizoram Science Congress
held at Mizoram University during 13th - 14th October 2016.

(Dr. R. LALFAKZUALA)
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Certificate

Certified that Mercy H. Lalramdinseli Participated / acted as a Resource person in the Workshop/ Training on National Level Workshop on Biostatistics and Bioinformatics held during 01-07 Sept. 2016 organized by Department of Biotechnology, Mizoram University sponsored by Bioinformatics Infrastructure Facility, Department of Biotechnology (DBT), New Delhi.

(Prof. R. Lalthantluanga)
Vice-Chancellor
Mizoram University

Professor
Department of Zoology
Mizoram University

(Prof. N. Senthil Kumar)
Coordinator, DBT-BIF Centre
Mizoram University


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CERTIFICATE OF ATTENDANCE
This is to certify that
Mercy H. Lalrawdinfeli
has attended
The Workshop on
Mechanisms of adaptation in the Temporal Environment
May 23, 2017
Organized at: Department of Zoology, Mizoram University, Aizawl, Mizoram
Prof. Vinod Kumar
President, InSC
Prof. G Gurusubramanian
Chairman
Prof. N. Senthil Kumar
Coordinator, State Biotech Hub
Dr. Amit Kumar Trivedi
Convener


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to

Mercy H Lalramdinfeli

in recognition of participation in the Science Communication Workshop (SciComm 101)

held on 6 June, 2017 at Mizoram University, Aizawl

Dr. Shahid Jameel
Chief Executive Officer

CERTIFICATE

THIS IS TO CERTIFY THAT

MERCY H. LALRAMDINFELI

has participated at Technical Session/ Exhibition in Mizoram Science Congress held at Mizoram University during 13th - 14th October, 2016

(Dr. R. LALFAKZUALA)	(Dr. LALDINPUIA)	(Dr. R.K. LALLIANTHANGA)
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Comparative study on the breeding and development of two rhacophorids, *Rhacophorus maximus* Günther, 1858 and *Polypedates teraiensis* (Dubois, 1987)

AN ABSTRACT SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF PHILOSOPHY IN ZOOLOGY

BY

MERCY H. LALRAMDINFELI

REGISTRATION NO: MZU/M.Phil./395 of dt.26.5.2017

UNDER THE SUPERVISION OF

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INTRODUCTION:

Out of 32 families the Rhacophoridae family constitutes a radiation of almost 409 tree frogs and allied species (Frost, 2017) with members distributed from Asia to Africa (Duellman, 1999).

Rhacophorus maximus commonly known as Nepal flying frog, Günther's tree frog, giant tree frog is listed as Least Concern by the IUCN Red List due to its extensive distribution and its tolerance of a wide range of habitat types (IUCN *et. al.,* 2006). It was described by Günther in 1858 from the type locality Nepal. It is a species of frog in the Rhacophoridae family found in southwestern China (Yunnan, Tibet), north eastern India, Nepal, western Thailand, and northern Vietnam, and possibly in Bangladesh. *Polypedates teraiensis* is known as the perching frog, six - lined tree frog, or Terai tree frog. It also a species of frog in the Rhacophoridae family which is found in eastern Nepal, eastern, peninsular, and north-Eastern India (West Bengal, Meghalaya, Mizoram, Assam, Arunachal Pradesh, Nagaland, Manipur, Sikkim, also reported for Gujarat and Madhya Pradesh) and Bangladesh, into adjacent Myanmar, and possibly into adjacent China.

Both *Rhacophorus maximus* and *Polypedates teraiensis* construct foam nest to protect the eggs and embryos from predation and desiccation (Heyer, 1969 and Downie, 1993).The social behaviors of most anurans are associated with acoustic communication in the form of vocalization (Krishna and Krishna, 2005). Advertisement calls attract females to breeding areas, and announce to other males that a given territory is occupied. Advertisement calls are species specific. Rainfall also fills the pools and ponds and provides excellent breeding sites for a number of anuran species, as there must be some standing water for their breeding activity. The diversity of reproductive modes in amphibians is higher than in any other vertebrate group (Caldwell, 1992). Once the males and females have found each other, mating can occur. 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). One of the most interesting modes is that of rhacophorids, where majority of them deposit their eggs in foam nests, while others exhibit direct development (Duellman and Trueb, 1994). Most eggs hatch into aquatic larvae i.e. tadpoles.

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). 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. The tadpoles than metamorphose into a froglet than into adult frog. Knowledge of food and feeding behavior of tadpoles is very essential as early part of life history of amphibian is dependent on availability of food items in natural habitat (Sinha *et. al.,* 2001).

REVIEW OF LITERATURE

Reviews of literature reveals that studies on the larval stages, breeding and nesting behavior of *Rhacophorus maximus* and *Polypedates teraiensis* has been done by few workers. Orlov *et al.,* (2008) mentioned the presence of *Rhacophorus maximus* in Vietnam but did not provide specimen based, specific locality records. Wildenheus *et. al.,* (2010) studied the larval and juvenile stages of *Rhacophorus maximus* occurs in Vietnam. Luu *et. al.,* (2014) also reported the occurrence of *Rhacophorus maxim*us for the first time from Laos and reported the morphological characters of the species, but did not provide about the breeding and developmental stages. Khongwir *et. al.,* (2016) reported the breeding and nesting behaviour of *Rhacophorus maximus* at Cherrapunjee and Mawsynram, Meghalaya, North East India. Sinha *et. al.,* (2001) conducted food spectrum analyses based on tadpoles of *Rhacophorus maximus* from India, and Khongwir *et. al.,* (2003) studied metamorphosis and the development of mouth region. Drawings of tadpoles from Nepal in an obviously early developmental stages were published by Anders and Rai, (2002) who also dealt with the advertisement call of the species. Chakravarty *et. al.,* (2011) from Assam have reported the tadpole morphology and the developmental stages of *Polypedates teraiensis*. Tamuly and Dey (2014) reported the larval morphology and development of *Poypedates teraiensis* in Assam, North East India. 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. 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.

Although recent research has been done on the natural history and larval morphology of *Rhacophorus maximus* and *Polypedates teraiensis*, information on the ecology of its habitat, feeding and breeding behavior during breeding season in Mizoram remains limited. The knowledge of larval adaptation is essential for adequate amphibian conservation measures, still there is scanty information on the breeding biology of these tree- frogs. (Khongwir *et. al.*, 2016). Therefore the present investigation was carried out for a better understanding of its breeding and developmental biology in Mizoram.

OBJECTIVES

The major objectives of the proposed study are as follows:

1. To study the habitat of *Rhacophorus maximus* and *Polypedates teraiensis*
2. To study the breeding behavior with respect to environmental factors.
3. To study the developmental stages with reference to morphometric changes of the tadpoles.
4. To study oral morphology, food and feeding behavior of tadpoles and adults of *Rhacophorus maximus* and *Polypedates teraiensis*

MATERIALS AND METHODS

i) Study sites: To study the breeding behavior, habit and habitat, and the environmental factors of the breeding sites of *Rhacophorus maximus* and *Polypedates teraiensis*, two study sites designated as study sites I and II were selected inside the campus of Mizoram University, Tanhril, Aizawl. Breeding behavior, amplecting pairs and freshly spawned eggs were studied and documented with the help of photographic and video cameras. The amplecting pairs and their newly laid were photographed with a camera, Sony Cyber-shot DSC-H10 (Super Steady Shot). The temperatures of atmospheric, water and relative humidity of the study sites were recorded by thermometer. Water pH were also measured with the help of pH pen (Hanna instrument).

ii) Acoustic analysis: Mating calls were recorded with the help of digital voice recorder Sony ICD-PX440 Proffesional compact voice recorder. The sampling used to convert the

signals to digital format was 8 KHz with 16–bit precision. The oscillogram was prepared and analyzed with the help of a software tool "SoundRuler Version 0.9.6.0 (acoustic analysis)". The data were analyzed with the help of statistical software tools SPSS (7.5.1 version) and OriginPro 8 SRO (8.0724 version).

iii) Development and metamorphosis: To study the development and metamorphosis, investigation were conducted in the natural environment (at the study site) as well as in the laboratory. Amplecting pairs and the freshly spawned eggs were brought to the laboratory and allowed to lay their eggs for further development and metamorphosis. Staging of the developmental stages were done on the basis of external morphological changes as per the criteria described by Gosner (1960). Photographs of the developmental stages were taken with the help of microscope with photographic attachments. The hatched tadpoles were reared in a plastic tray containing water collected from the study sites. The temperature in the laboratory conditions were monitored in order to know the stages attain by the developing tadpoles. The rate of development were observed with the help of a stereoscopic dissecting binocular microscope during field observations and in the laboratory condition. Water temperature and pH were recorded during the development of embryo and larvae.

iv) Morphometric measurement: Measurements of the frogs were carried out using a dial caliper accurate to 0.02 mm. Morphometric measurements largely follow the combination of Chanda (1994), Bain *et. al.*, (2006) and Ohler (2007). Morphometric measurement of the tadpoles were also taken using a dial caliper following the method of Altig (2007). Different stages were categorized into Operculum, oral discs and pigmentation, hind limb bud development, toe differentiation and metamorphic stage as per given by Mc Diarmid and Altig, 1999. Five number of tadpoles (N=5) were measured in different developmental stages.

v) Food and feeding behaviors in relation to their oral structures: To study the food and feeding behavior of the tadpoles, different developmental stages of the tadpoles were collected and preserved in 4% formaldehyde and autopsied for qualitative analysis of the gut contents. All food items were expressed in terms of percent abundance and percent frequency of occurrence. The degree of dominance of food items and the species diversity in

stomach sample, Berger- Parker Diversity Index and Shannon –Wiener Index were calculated respectively, and the species were also calculated. Identification on the food items of the tadpoles were made following the methods of Edmonson (1959) and Smith (1994). Food items were identified upto genus level. The oral structures of the tadpoles were also studied using stereoscopic binocular microscope (CETI 9554. 1200) following the criteria of Altig and Mc Diarmid (1999) and Altig (2007). Description of oral disc and labial tooth raw formula (LTRF) were also made according to the method done by Altig (2007).

SUMMARY OF RESULTS

- The present study provides informations about the breeding behavior, food and feeding behavior and development of two sympatric rhacophorids, *Rhacophorus maximus* and *Polypedates teraiensis.*
- The two study sites were found to be excellent breeding grounds and served as good habitat for the development and metamorphosis of this two sympatric species.
- It was observed that *Rhacophorus maximus* is a seasonal breeder and its breeding activity coincides with the onset of monsoon season i.e., February to April.
- Advertisement call of *Rhacophorus maximus* was strongly pulsed, short, consisting of 2 pulses, and have frequency spectra with a dominant band of 1464.258 Hz and the band width ranges from 367.1037 to 692.2985 Hz.
- The water temperature for breeding and completion of life cycle in *Rhacophorus maximus* ranged between 21ºC to 30ºC, pH between 5.14- 8.75.
- The breeding activity of *Polypedates teraiensis* was observed from March to August.
- The advertisement call of *Polypedates teraiensis* was short consisting of a single pulse, and have frequency spectra with a dominant band of 1981.005 Hz and the band width ranges from 359.5705 to 1570.255 Hz.
- The atmospheric temperature recorded for breeding and completion life cycle of *Polypedates teraiensis* ranges between 26ºC to 38ºC and the water temperature ranges from 24ºC to 28ºC, pH between 5.54 - 8.22.
- Distinct sexual dimorphism was observed in both the species where females are larger than males. Snout Vent Length (SVL) of amplecting females of *Rhacophorus maximus* ranges from 66.48 - 76.90 mm and from 67.89 - 75.22 mm in *Polypedates teraiensis,*

whereas the SVL of males ranges from 47.88 - 70.14 mm in *Rhacophorus maximus* and from 52.93 - 56.45 mm in *Polypedates teraiensis*.

- The duration for the development and completion of life cycle in *Rhacophorus maximus* was 52 - 53 days whereas within 61 - 64 days in *Polypedates teraiensis*.
- The size of the eggs of *Rhacophorus maximus* measures 2.15±0.07 mm (N=5) while eggs of *Polypedates teraiensis* measures 1.34±0.07 mm (N=5).
- The embryo of *Rhacophorus maximus* start hatching at Gosner stage 21(11.57 ± 0.25 mm) and at stage 20 (7.18 ±0.49 mm) in *Polypedates teraiensis.*
- In *Rhacophorus maximus,* the total body length of the tadpoles was maximum (50.34 ± 0.59 mm) at stage 39 and in *Polypedates teraiensis.*
- In both the species, the right side of the forelimb emerged first followed by the left side of the limb at stage 42. At stage 44, tail was greatly reduced and metamorphosis was completed at stage 46.

- Study observed that tadpoles start feeding from stage 25 onwards in both the species.
- During the study period, hervivorous behavior was observed as the tadpoles feed on plants and algae, and cannibalism was also found among the two species.
- The tadpoles soon stop feeding at stage 42 and from stage 46 onwards the froglets feed on carnivorous diet.
- Analysis of the intestinal contents of the tadpoles revealed that *Polycystis* belonging to the genus Chlorophyceae were the most dominant food items in the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis.*
- The stomach contents of both adults *Rhacophorus maximus* and *Polypedates teraiensis* were more or less similar. It was observed that the adult frogs consumed termites, beetles, dipterans, and small orthopterans, and pieces of leaves and twigs were also found in both the species.
- Observation of the oral structures of the tadpoles of *Rhacophorus maximus* and *Polypedates teraiensis* reveals that the oral discs are positioned antero - ventrally and slightly emarginated, and was composed of anterior (upper) labium and posterior (lower) labium.

- The jaw sheath of *Rhacophorus maximus* was dark brown and serrated with a lower jaw sheath curved and with long appendices, whereas, serrated jaw sheath with a lower jaw sheath large arch with weak median convexity of *Polypedates teraiensis* were observed.
- The upper jaw sheaths were found to be V – shaped and the mouth was present in between in both species.
- LTRF of *Rhacophorus maximus* was 3(2 - 3)/3(1) at stage 25, 4(2 - 4)/3(1) at stage 26 - 29 and 5(2 - 5)/3(1) from stage 30 onwards, whereas, LTRF of *Polypedates teraiensis* was 3(2 - 3)/3(1) at stage 25 and from stage 26 onwards the tooth row formula was consistent at 4(2 - 4)/3(1) upto stage 41.
- Study shows that ontogeny i.e., changes in larval oral structure was observed in both the species.
- Their oral structures starts to degenerate at stage 42 and gradually transformed into adult mouth and from stage 46 onwards, the froglet starts feeding on a carnivorous diet.
- From the present investigation, it was observed among the two species that the length of intestines of the tadpoles start increasing from stage 25 to 40, and there is a significant positive correlation between the Gosner stages from stage 25 to stage 40, where $p<0.01$ at the 0.01 level (2-tailed).
- In *Rhacophorus maximus*, from stage 41 - 46, the length of the tadpoles were greatly reduced, showing significant positive correlation between Gosner stages with that of the total length and gut length where $p = 0.016$ at 0.05 level (2-tailed).
- In *Polypedates teraiensis*, the length of the tadpoles also reduced which reveals that there is a positive correlation between developmental stages with that of the total length and the gut length where p□ 0.01 at 0.01 level (2-tailed).
- In both *Rhacophorus maximus* and *Polypedates teraiensis*, it was observed that as the tadpoles continue to grow, so does the intestine and as the tadpoles regresses from stage 41 onwards, intestine also begins to shorten to modified for carnivorous feeding behavior.
- This study contributes on the information of breeding behavior, habit and habitat, development, food and feeding behavior of these two rhacophorids, *Rhacophorus maximus* and *Polypedates teraiensis* prevalent in Mizoram, can be useful for their conservation measures.