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Oviposition sites of some Anurans in East Khasi Hills, Meghalaya, North East India

Abstract

We investigated the oviposition sites and breeding period of 13 species of anurans with special reference to East Khasi Hills of Meghalaya, North Eastern India during the period 2004 - 2015. The 13 species included *Amolops assamensis, Euphlyctis cyanophlyctis, Fejervarya teraiensis, Hyla annectans, Kaloula pulchra, Leptolalax khasiorum, Odorrana livida, Odorrana mawphlangensis, Polypedates himalayensis, Polypedates teraiensis, Rhacophorus bipunctatus, Rhacophorus maximus* and *Xenophrys parva*. Our study therefore identifies the different types of oviposition sites and breeding habitats of the anuran amphibians and helps to understand the importance of such sites. This in turn may help to monitor proper management on preserving such habitats for protecting the amphibian as these animals are facing threats of extinction due to habitat loss and degradation.

Key words: *Amphibian, breeding habitats, oviposition sites, habitat loss, conservation.*

Introduction

Oviposition site selection is an important factor that affects the reproductive success of anuran amphibians which breed in a wide variety of aquatic habitats. An appropriate choice of oviposition site is especially critical for oviparous animals that lack parental care (Murphy 2003). For these animals, suitable oviposition sites should protect vulnerable eggs from hydric and thermal stress, predators, and parasites. Several studies indicate that both abiotic and biotic characteristics of the breeding site play an important role in influencing the adult females to choose a potential oviposition site which in turn determines the survival of the offspring (Wells 1977a). Parental care by the adult frogs enhances the survivorship and fecundity of the offspring (McDiarmid 1978). However, in organisms which lack parental care, the survival and growth of the offspring may depend on the quality of the habitat in which their eggs are deposited. Thus, adult frogs are expected to choose habitats that maximize their fitness when potential habitats vary in their suitability for juveniles. Deposition of eggs by females in unsuitable habitats results in fewer offsprings survival than females that oviposit in suitable habitats; selection of oviposition site by females depends strongly on selective pressure to maximize offspring survival (Crump 1991).

Cherrapunjee plateau of Meghalaya provides ideal breeding habitat for amphibians. Photo Credit: Abhijit Das

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Hyla annectans.
Photo Credit: Abhijit Das

Meghalaya state in North East India is a part of the Global biodiversity hotspot. Amphibian research from this region is largely included survey and taxonomy (Roonwal and Kripalani 1961; Yazdani and Chanda 1971; Pillai and Yazdani 1973; Pillai and Chanda 1977, 1979; Sahu and Khare 1983; Hooroo et al. 2002; Sen 2004; Rangad et al. 2007; Das et al. 2009 and 2010) and few on breeding biology and development (Roy 1979; Khongwir 2004, 2016; Iangrai 2007; Rangad 2014 and Tron 2014). The present study emphasizes the importance of the breeding habitat and oviposition site of these anurans in Meghalaya. Obtaining accurate information on amphibian breeding habitats and oviposition sites can be challenging as many species have different breeding biology requiring different habitat for breeding and oviposition. Presently, amphibian species all over the world are experiencing significant threats extinction due to habitat loss and degradation. In East Khasi Hills of Meghalaya the major forms of habitat destruction are in the form of mining (sand, limestone and coal), quarrying, constructions and forest fires. Identifying their breeding habitats and oviposition sites has now become necessary to understand how these changes affect habitat suitability for different anuran species. Proper management targeted at enhancing amphibian breeding areas and oviposition sites can help in protection and conservation of the amphibian species.

Materials and Methods

Survey was conducted in different areas of East Khasi Hills, Meghalaya, North Eastern India from the year 2004 – 2015. The location (latitude/longitude) and elevation of the surveyed areas were determined with the help of Garmin (etrex) Global Positioning System (GPS). Survey was conducted in the afternoon from 2:00 PM and continued till evening depending on their breeding season. After identification of the sampling site, survey period at each selected site was covered at different times of the day in order to record the breeding period of the selected species. All the species have been identified by comparing their measurements and morphological characters with the description of their respective holotype and available published literatures (Chanda 1994; Sengupta et al. 2008; Das et al. 2010). Audio Encounter Surveys (AES) and Visual Encounter Surveys (VES) were used to identify exact locations where adult frogs are attempting to breed. Egg mass and larval surveys provide evidence about the oviposition site and the breeding period of the species. Torch light, headlamp and bamboo torch were used to locate and count the number of males and females of the frogs at their breeding sites during night surveys. Field sampling was carried out daily during the

breeding season and at weekly intervals during the non- breeding period with each sampling session spanning over two to three continuous days. All photographic documentation was made using digital SLR (Nikon D 3200).

Results

Oviposition sites of the 13 anuran species of East Khasi Hills (*Amolops assamensis, Euphlyctis cyanophlyctis, Fejervarya teraiensis, Hyla annectans, Kaloula pulchra, Leptolalax khasiorum, Odorrana livida, Odorrana mawphlangensis, Polypedates himalayensis, Polypedates teraiensis, Rhacophorus bipunctatus, Rhacophorus maximus* and *Xenophrys parva*) were observed (Table 1) during the study from different types of breeding habitats and a description of the oviposition site of each anuran species is described below:

Oviposition site of *Amolops assamensis*

The oviposition site of *Amolops assamensis* (Figure 1a) was observed in a fresh water stream in Nongspung village (25°27' N; 91°36' E; 1644m.asl) located about 50 km. away from Shillong. *Amolops assamensis* a large frog where snout vent length ranges from 53-90 mm (Sengupta et al. 2008) breeds during the months of February to March in a flowing stream a short distance away from Nongspung village. This species was first described by Sengupta et al. (2008) from the splash zones of moist dark crevices in a fast flowing stream in Assam. It was observed that the adults of *Amolops assamensis* emerge as early as in the month of January and they come to breed in the same spot of the stream every year during the study period (2009-2012). The oviposition site was observed to be on the

Figure 1a : A fresh water stream in Nongspung village. Meghalaya

Figure 1b : The oviposition site of *Amolops assamensis*.

Figure 1C : The female *Amolops assamensis* lay egg masses that are attached to the surface of the rock walls in the breeding habitat

side of a flowing stream that cascades into a deep pool (Figure 1b). This stream was lined with rocks and pebbles, mostly covered by green moss where the frogs come out to breed. Adults have been observed to show aggregation behaviour during the breeding period. The females lay egg masses that are attached to the surface of the rock walls in the breeding habitat about 2 to 3 feet below the water (Figure 1c).

Oviposition site of *Euphlyctis cyanophlyctis*

Oviposition sites of Indian skipper frog, *Euphlyctis cyanophlyctis* (SVL 31-61 mm) (Figure 2a) found to be temporary and permanent water bodies such as, ponds, muddy pools and marshes of Cherrapunjee (25°18?N; 91°42?E; 1484m.asl), Mylliem (25°30?N; 91°49?E; 1625m.asl) and NEHU campus, Shillong (25°36'N; 91°53'E; 1418m.asl) (Figure 2b). It is a seasonal breeder and its breeding activity coincides with the monsoon season i.e. March up to August. In addition, it was also observed that the frog would deposit its eggs on floating algae and among submerged vegetation on the edges of standing water bodies (Figure 2c). The eggs deposited were scattered and spread over the water surface as depicted. *Euphlyctis cyanophlyctis* exhibits aquatic oviposition as the eggs are laid in the water body.

Figure 2a : Indian skipper frog, *Euphlyctis cyanophlyctis* adult male.

Figure 2b : Habitat of Indian skipper frog, *Euphlyctis cyanophlyctis*.

Figure 2 c : Eggs deposited by Indian skipper frog, *Euphlyctis cyanophlyctis*.

Oviposition site of *Fejervarya teraiensis*

The oviposition of *Fejervarya teraiensis* (SVL 35.7-52.3 mm) (Figure 3a) was documented at Malki forest, Shillong (25°35'N; 91°55'E; 1500-1800m.asl) and Laitkroh community forest (25°26'N; 91°48'E; 1610m.asl). *Fejervarya teraiensis* found to select rain fed pools that are permanent or semi permanent, rain water puddles, marshes and ditches (Figure 3b). *Fejervarya teraiensis* breeds throughout the months of April to July. The oviposition sites are surrounded by vegetation which provides ideal hiding place for the adult frogs. Breeding activity starts once the pools and pond gets filled with rain water. The females deposit their eggs in these aforementioned sites and the eggs can be found floating on the surface of the water (Figure 3c). The eggs are pigmented and have a thin layer of jelly cover. The tadpoles were observed to swim freely in the water column or were swimming close to margin of the ponds (Figure 3d).

Figure 3 a : Amplexus in *Fejervarya teraiensis*

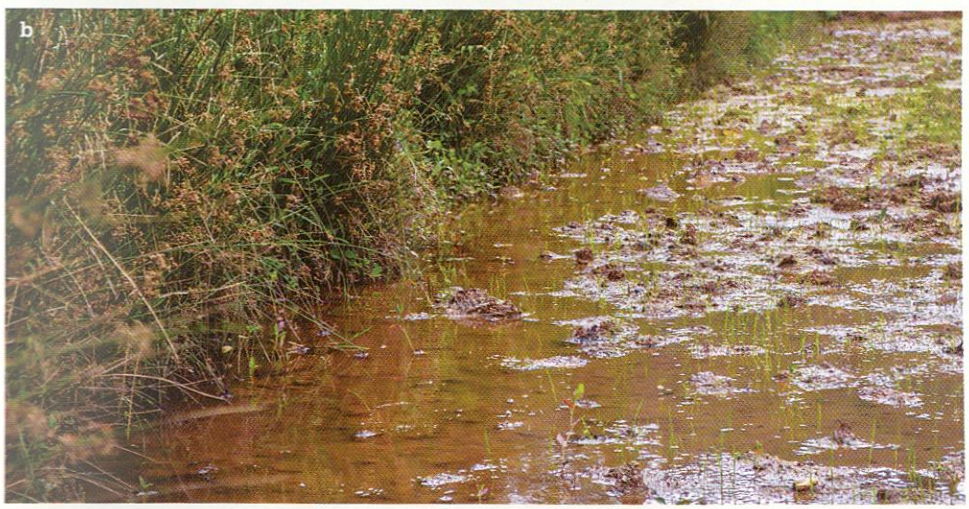


Figure 3 b : Habitat of *Fejervarya teraiensis*

Figure 3c : Eggs deposited by female of *Fejervarya teraiensis*.

Figure 3d : Tadpoles of *Fejervarya teraiensis*.

Oviposition site of *Hyla annectans*

Hyla annectans (SVL 23-48 mm) (Figure 4a), the Indian hylid frog was collected from Mylliem (25°30'N; 91°49'E; 1625m.asl) as well as the grassland areas of Cherrapunjee (25°18'N; 91°42'E; 1484m.asl). In Meghalaya, *Hyla annectans* found to breed during the month of March till June during the early part of the monsoon season. However, Ao and Bordoloi (2000) reported that this species breeds during the month of May to July in Nagaland. In the present study it was observed that this species breeds in temporary ponds, rainfed pools, puddles and waterlogged terraced paddy fields at forest edges (Figure 4b). A unique feature about this frog is that it selects its oviposition site only on pristine and clean water bodies as was observed in the instant case in the forest edges which are free from any anthropogenic disturbances. Adult female of *Hyla annectans* chooses to oviposit its eggs on submerged vegetation (Figure 4c). The eggs which are covered with thick jelly stick to one another and float as a mass on the water. *Hyla annectans* thus shows aquatic oviposition which is a feature of most anuran amphibians.

Figure 4a : Amplexus in *Hyla annectans*.

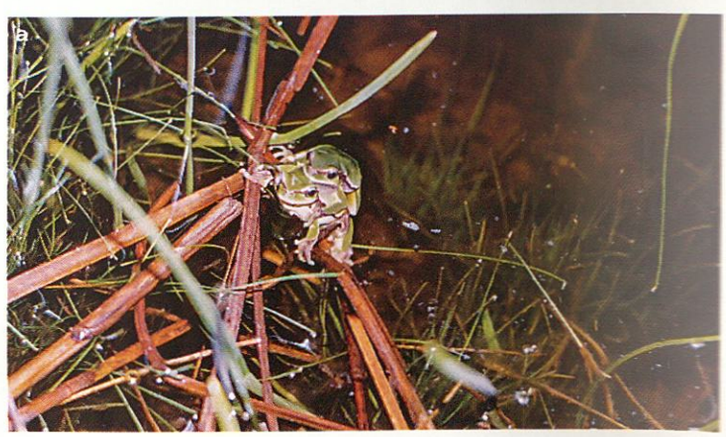


Figure 4b : Habitat of *Hyla annectans.*

Figure 4c : Eggs deposited by female *Hyla annectans*

Oviposition site of *Kaloula pulchra*

During the present survey, the oviposition site of *Kaloula pulchra* (SVL 60.5-64.21 mm) (Figure 5a) was studied at Laitkynsew village (25°13'N; 91°39'E; 915m.asl) and Cherrapunjee (25°18'N; 91°42'E; 1484m.asl) East Khasi Hills, Meghalaya. *Kaloula pulchra* select standing water bodies like cemented tanks, rock pools, rain fed pools as their breeding habitats (Figure 5b) during the months of April to July. *Kaloula pulchra* was commonly found in artificial tanks or wells which are used by the villagers for either storing water or most usually for fermenting "kwai" or the betel nut (*Areca catechu*) (Figure 5c). During the monsoon period these tanks or wells get filled with water. The villagers dip sacks of Areca catechu in these wells for months and leave them there undisturbed. In such water tanks, the adult frogs of *Kaloula pulchra* were found to deposit their eggs on the surface of the water. These pigmented eggs which are covered with thin jelly covering float on the water surface.

Figure 5a: Oviposition site of *kaloula pulchra.*

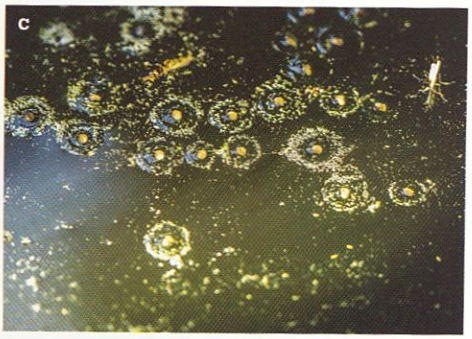


Figure 5b: Breeding habitat of *kaloula pulchra.*

Figure 5c: Eggs in the habitat of *Leptolalux khisiogum*

Oviposition site of *Leptolalax khasiorum*

Das et al. (2010) described *Leptolalax khasiorum* (SVL 25.36-31.89 mm) from a forest stream in Mawphlang (25°26'N; 91°44'E; 1813m.asl). Tron et al. (2015) observed that the breeding period of this frog lasts for a very short time i.e. from the month of February to March (Figure 6a) and adults can be found till the month of April. It was observed and reported that the adult frogs prefer to remain hiding under rocks and are found after lifting rocks along the stream bed during daytime. Since these frogs emerge in early spring, sometimes even before the rain sets in, the only choice as breeding areas are mostly confined to small pockets of water or puddles in the rocky stream bed (Figure 6b). These water pools are rich in organic debris derived from leaf litter along with mud and sand. Females lay egg masses that are usually attached to rocks, leaves sometimes even

on the underside of rocks in the stream bed and on the edges of the stream (Tron et al. 2015) (Figure 6c). Eggs are small and white and covered with a thin transparent jelly (Figure 6d).

Figure 6a: *Leptolalax khasiorum* in its habitat.

Figure 6b: Habitat of *Leptolalax khasiorum.*

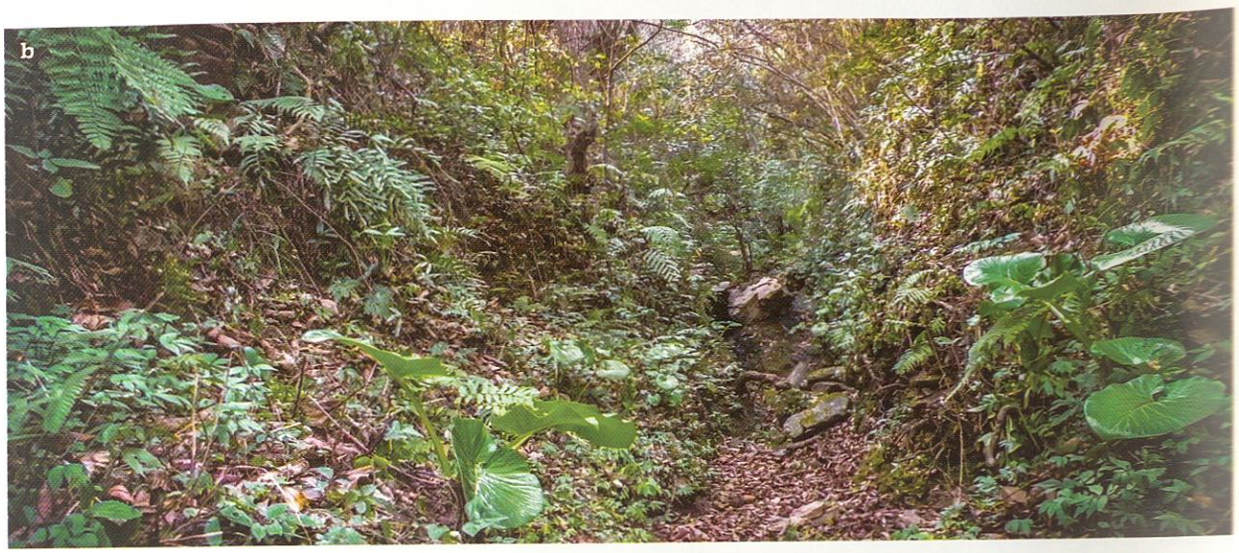


Figure 6c, 6d: Habitat of *Leptolalax khasiorum.*

Oviposition site of *Odorrana livida*

Observation on oviposition of *Odorrana livida* (SVL 68.63- 97.44 mm) was made from forest streams at Riat Laban (25°36'N; 91°53'E; 1500-1800 m.asl) and Malki forest (25°35'N; 91°55'E; 1500-1800m.asl). Adults of *Odorrana livida* (Figure 7a) have been seen to emerge in the breeding habitat during the months of May to July. The adults have been observed to breed in stream section having small collection of stagnant water pool surrounded by riparian vegetation. The stream bank was observed to have earth or rock walls with small crevices in them, from where the adult frogs have frequently been seen to emerge (Figure 7b). These frogs do not breed randomly throughout the stream. Females lay egg masses that are submerged in water along the side of the stream and in some cases attached to wet rocks and pebbles along the forest stream (Figure 7c). The eggs are individually covered with jelly layers and are attached to each other. The eggs form a multitier layer to form a clump.

Figure 7a: *Odorrana livida* in its Habitat

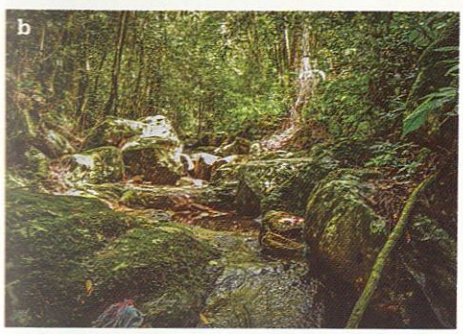


Figure 7b: Habitat of *Odorrana livida*.

Figure 7c: Egg deposition site of *Odorrana livida*.

Oviposition site of *Odorrana mawphlangensis*

The oviposition site of *Odorrana mawphlangensis* (SVL 61.93-94.97 mm) was studied in a forest stream at Mawphlang (25°26'N; 91°44' E; 1830m.asl). *Odorrana mawphlangensis* (Figure 8a) was observed to start emerging in the habitat from late April onwards. Their breeding activity starts from May and last till July or early August. It was generally observed that the calling males of this species start emerging first. *Odorrana mawphlangensis* used specific oviposition sites in the stream during the period of investigation (Figure 8b). The oviposition site is a pool of water with a soft substratum of mud and organic debris. The selected stream had half submerged boulders that formed cave-like spaces which were used as oviposition sites (Figure 8c). These specific sites are seen at dark and damp places with a canopy cover. Adults have been observed to emerge from the holes on the sides of streams about 1.5 to 3 metres above the stream near the oviposition site. The female usually lays egg masses directly on the substratum of the stream or attached to rock walls on the side of the stream and the egg mass is always submerged inside the water (Figure 8c). It was observed that each of the individual egg is wrapped in a semi-transparent jelly in the egg masses.

Figure 8a: *Odorrana mawphlangensis* in its Habitat.

Figure 8b: habitat of *Odorrana mawphlangensis*.

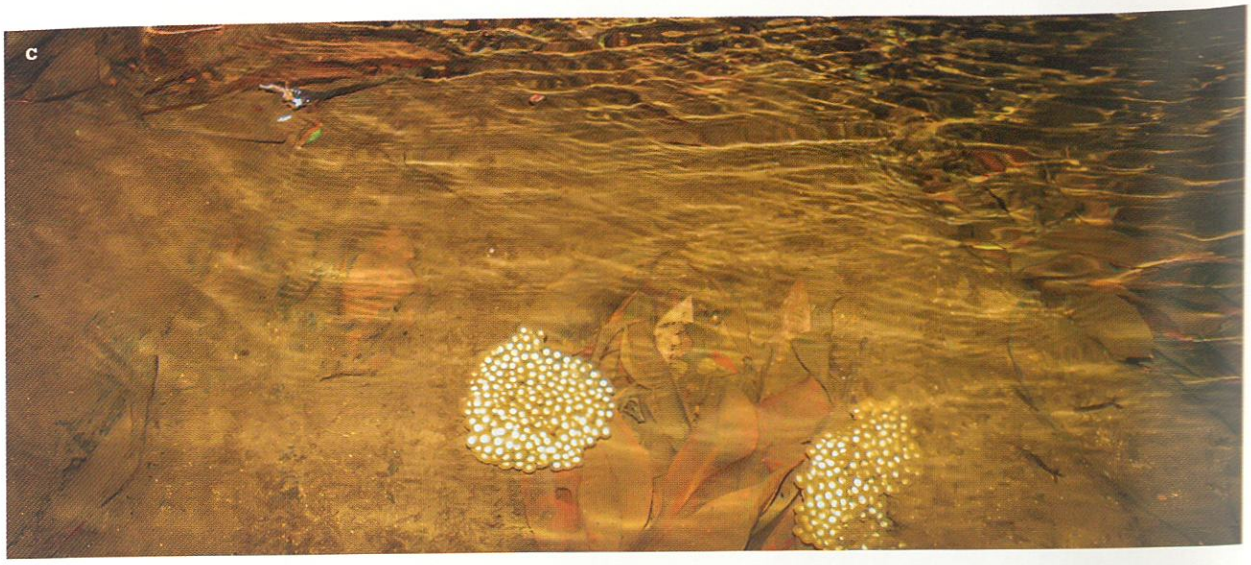


Figure 8c : Egg deposition site of *Odorrana mawphlangensis.*

Oviposition site of *Polypedates himalayensis*

During the present investigation, it was observed that *Polypedates himalayensis* (SVL 41.0- 62.0 mm) (Figure 9a) is a seasonal breeder and its breeding activity coincides with the onset of few showers of rainfall i.e. from March to July. The study was conducted at an agricultural field (Figure 9b) which is adjacent to a community forest located at a village named Mylliem (25°30'N; 91°49'E; 1625m.asl) about 18 km. from Shillong, East Khasi Hills District. During the study, it was found that *Polypedates himalayensis* selects its oviposition site near standing water bodies. Females of *Polypedates himalayensis* lay its eggs by constructing foam nests half a meter or in some cases even one meter away from waterbody (Figure 9c). The construction of foam nest was found to occur inside earthen holes which are damp, moist and also under thick vegetation cover.

Foam nests of *Polypedates himalayensis* were also collected from a pond located at North Eastern Hill University (NEHU). In such built up habitats *Polypedates himalayensis* constructs foam nests under moist or wet planks, logs of wood and also under vegetation (pine leaves of *Pinus khasiana*) (Figure 9d). Spawning and foam nest construction started from the month of April and was seen to last till the month of July.

Figure 9a : Amplexus in *Polypedates himalayensis.*

Figure 9b : Habitat of *Polypedates himalayensis.*

Figure 9c : Females of *Polypedates himalayensis* lay its eggs by constructing foam nests nearby waterbody.

Figure 9d : *Polypedates himalayensis* constructs foam nests under moist or wet planks, logs of wood and also under vegetation.

Oviposition site of *Polypedates teraiensis*

Polypedates teraiensis (SVL 42.0-78.0mm) (Figure 10a) or the common tree frog is a seasonal breeder and its breeding activity coincides with the onset of monsoon. The breeding period of this species was found to last from March to early September. In the present study, it was seen that *Polypedates teraiensis* is commonly found in many grassland pools located at Cherrapunjee (25°18'N; 91°42'E; 1484m.asl) and many foam nests were observed in these grassland pools during the breeding season (Figure 10b). Further it was observed that *P. teraiensis* is syntopic with *P. himalayensis*. This tree frog selects its oviposition sites close to water bodies and sometimes lays its eggs in vegetation cover above water. In this case the oviposition is aquatic and it occurs in standing water of the grassland pools. Apart from construction of foam nests in grassland vegetation, the study also revealed that *Polypedates teraiensis* sometimes chooses to construct its foam nest in cemented tanks about 15-30 cm above water in Cherrapunjee (Figure 10c) and also on small rocks and stones on or near temporary pools (Figure 10d).

Figure 10a : Amplexus observed in *Polypedates teraiensis*.

Figure 10b : Foam of *Polypedates teraiensis* nests were observed in these grassland pool.

Figure 10c : *Polypedates teraiensis* sometimes chooses to construct its foam nest in cemented tank.

Figure 10d : Eggs deposited by *Polypedates teraiensis* stones on or near temporary pools

Oviposition site of *Rhacophorus bipunctatus*

Rhacophorus bipunctatus (SVL 39.0-65.0 mm) (Figure 11a) or the twin spotted tree frog is also a seasonal breeder with its breeding period coinciding with the onset of monsoon i.e. from the month of March to August. The present study recorded the occurrence of this species from Cherrapunjee, Laitkynsew Village (25°13′ N; 91°39′ E; 915m.asl) and NEHU Campus Shillong. During the present investigation, it was found that *Rhacophorus bipunctatus* chooses its oviposition sites on overhanging vegetation above water bodies (Figure 11b). It was observed that this frog constructs small foam nests (6-8 cm in diameter) on leaves of trees, bushes and grasses that are close to about 1 m approximately above water bodies (Figure 11 c). Oviposition sites were also documented from tree holes and walls of cemented water tanks at Laitkynsew village (Figure 11 d). Apart from selecting overhanging vegetation to deposit its eggs, *Rhacophorus bipunctatus* also construct its foam nests on the ground surface covered by grasses or leafy vegetation either close to a water body or away from it.

Figure 11a: Amplexus observed in *Rhacophorus bipunctatus*.

Figure 11b: Egg deposition site of *Rhacophorus bipunctatus*.

Figure 11c: Egg deposition site of *Rhacophorus bipunctatus*.

Figure 11d: : *Egg deposition site of Rhacophorus bipunctatus.*

Oviposition site of *Rhacophorus maximus*

Rhacophorus maximus (SVL 35.0- 68.0 mm) (Figure 12a), the large tree frog was found in many grasslands and forest covers of Cherrapunjee (25°16 N; 91°44 E; 1484m.asl) as well as Mawsynram village (25° 18' N; 91° 35' E; 1400m.asl). Khongwir et al. (2016) reported that the breeding period begins with the onset of first shower of rainfall during the month of March and lasts till early May. This tree frog selects it oviposition site on grassy vegetation near shallow temporary rainfed pools and the female constructs large foam nests on such vegetation which then appears to be floating on the water surface (Figure 12b and 12c). In addition *Rhacophorus maximus* also chooses to oviposit its eggs on vegetation above water surface, on earthen banks attached to grasses and stones on the edges of the pond (Figure 12d). Such temporary rainfed standing water serves as a potential breeding site for this frog and since these temporary pools dry up fast so also the breeding period of this frog is also very short. Therefore, from the study it is clear that *Rhacophorus maximus* exhibits aquatic oviposition and constructs foam nests on any substrata where water is available.

Figure 12a: An adult individual of *Rhacophorus maximus* documented from cherapunjee, Mwghalaya.

Figure 12b: Female *Rhacophorus maximus* is laying eggs in the edges of the temporary pools.

Figure 12c: Foam nest of *Rhacophorus maximus* near pool edge.

Figure 12d: *Rhacophorus maximus* also chooses to oviposit its eggs on vegetation above water surface, on earthen banks attached to grasses and stones on the edges of the pond.

Oviposition site of *Xenophrys parva*

From this study it was observed that *Xenophrys parva* (SVL 31.62-39.27 mm) (Figure 13a) is a monsoon breeder that comes out to breed during the months of April to July. The breeding habitat of *Xenophrys parva* at Mawphlang (25°26'N; 91°44'E; 1814m.asl) is a forest stream (Figure 13b). Since this species emerges during the monsoon, there is abundant water in the stream around the time they emerge. Shallow pools are formed along the side of the stream with sand, stone and organic debris on the stream bed. The stream has sloping earth walls on both sides of the banks with rocks and boulders present along the stream. The vegetation growing along riparian area creates shaded pool with floating and submerged organic debris such as leaves, pollen, flowers, etc. *Xenophrys parva* has been observed to be found in the portions of the stream that is associated to vegetation growing close to the stream and sometimes overhanging it. The females of *Xenophrys parva* chooses to oviposit its eggs on the moist decaying leaf litter and organic debris present along the edges of the stream as well as on small stones and pebbles adjacent to the stream (Figure 13c and 13d).

Figure 13a: An adult individual of *Xenophrys parva* documented from Mawphlang, Meghalaya.

Figure 13b: Habitat of *Xenophrys parva*.

Figure 13c: The females of *Xenophrys parva* chooses to oviposit its eggs on the moist decaying leaf litter and organic debris present along the edges of the stream as well as on small stones and pebbles adjacent to the stream.

Figure 13d: Egg laying site of *Xenophrys parva*.

Table 1 : Anuran species recorded from different areas along with their breeding habitats and oviposition sites.

Anuran Species	Area	GPS location	Altitude (m.asl)	Breeding Habitats	Oviposition Sites
Amolops assamensis (Family- Ranidae)	Nongspung	25°27'N; 91°36'E	1644	Flowing stream	Lays eggs inside the water body.
Euphlyctis cyanophlyctis (Family-Dicroglossidae)	Cherrapunjee Mylliem NEHU Campus Shillong	25°18'N; 91°42'E 25°30'N; 91°49'E 25°36'N; 91°53'E	1484 1625 1418	Temporary as well as permanent standing water bodies, marshes, ponds and puddles.	Deposits eggs amidst aquatic plants on the muddy pools and marshes also on floating algae present on the edges of standing water bodies.
Fejervarya teraiensis (Family- Dicroglossidae)	Malki Forest; Laitkroh community forest	25°35'N; 91°55'E 25°26'N; 91°48'E	1500-1800 1610	Permanent or semi permanent habitat with a lentic ecosystem like a pond, rain water puddles.	Lays eggs on water surface.
Hyla annectans (Family-Hylidae)	Mylliem Cherrapunjee	25°30'N; 91°49'E 25°18'N; 91°42'E	1625 1484	Temporary ponds, rainfed pools, puddles and terraced paddy fields located at the edge of forests where water logging is observed.	Oviposit eggs on vegetation that is submerged in water
Kaloula pulchra (Family- Microhylidae)	Laitkynsew village Cherrapunjee	25°13'N; 91°39'E 25°18'N; 91°42'E	915 1484	Non-permanent and standing water bodies like cemented tanks, rock pools and rain fed pools.	Eggs deposited on the water surface.
Leptolalax khasiorum (Family- Megophryidae)	Mawphlang	25°26'N; 91°44'E	1813	Forest stream, breeding areas mostly confined to small pockets	Females lay egg masses that are usually attached to rocks or leaves, sometimes even

Anuran Species	Area	GPS location	Altitude (m.asl)	Breeding Habitats	Oviposition Sites
				of water or puddles that remains in rocky stream bed.	on the underside of rocks.
Odorrana livida (Family- Ranidae)	Riat Laban Reserve Forest	25°36'N; 91°53'E	1500-1800	Fast flowing forest stream, cascades.	Eggs laid are submerged in water and in some cases attached to wet rocks and pebbles along the forest stream.
	Malki Forest	25°35'N; 91°55'E			
Odorrana mawphlangensis (Family- Ranidae)	Mawphlang Sacred Grove	25°26'N; 91°44' E	1830	Forest stream.	Lay egg masses directly on the substratum or attached to rock walls and the egg masses are always submerged inside the water.
Polypedates himalayensis (Family- Rhacophoridae)	Mylliem	25°30?N; 91°49?E	1625	Agricultural field, perennial pond.	Lays eggs by constructing foam nests away from the water body, in earthen holes, under wet planks and covered vegetation.
	NEHU Campus Shillong	25°36'N; 91°53'E	1418		
Polypedates teraiensis (Family- Rhacophoridae)	Cherrapunjee	25°18?N;91°42?E	1484	Grassland pools, rain fed pools and cemented tanks.	Foam nest construction on vegetation above water surface, on surfaces of rocks close to water body and walls of water tanks.
Rhacophorus bipunctatus (Family- Rhacophoridae)	Cherrapunjee	25°18'N;91°42'E	1484	Forest, bushes, tree holes, water tanks, rainfed pools.	Constructs small foam nests on leaves of trees, bushes, grasses and tree holes that are close to or few meters above water bodies and also in walls of cemented water tanks.
	Laitkynsew village	25°13'N; 91°39'E	915		
Rhacophorus maximus (Family- Rhacophoridae)	Cherrapunjee	25°16'N;91°44?E	1484	Temporary shallow rainfed pools	Constructs foam nest on grassy vegetation that has been covered with water, on earthen banks attached to grasses and stones and any substrata where water is available.
	Mawsynram	25° 18' N;91° 35' E	1400	Temporary rainfed pond	
Xenophrys parva (Family- Megophryidae)	Mawphlang	25°26'N; 91°44'E	1814	Edges of forest stream accumulated with moist leave litter, organic debris, stones and pebbles.	Oviposition takes place on moist decaying leave litter and organic debris present along the edges of forest stream as well as on small stones and pebbles adjacent to the stream.

Discussion

Anuran amphibians occupy and deposit their eggs in diverse habitats (Resetarits Jr. 1996) Many anuran species require water for successful oviposition. The diversity of aquatic habitats in which anurans can choose, range from shallow pools, rainfed puddles, cemented tanks, small temporary ponds to large permanent ponds, lakes, mountain streams, and rivers. Anurans clearly show selective preference for different habitats for oviposition. Anuran species that typically breeds in standing water is unlikely to oviposit its eggs in fast flowing stream. Different anuran species are expected to exhibit active selection of oviposition site rather than deposit their eggs in the environment at random, because offspring survival is strongly dependent on characteristics of oviposition site. These characteristics include physical parameters such as water depth (Crump 1991), size of the site, water temperature (Herreid and Kinney 1967; Howard 1978, 1980; Seale 1982; Waldman 1982; Caldwell 1986), and vegetation structure (Wells 1977b and Howard 1978), as well as biotic features such as potential predators and competitors (Resetarits and Wilbur 1989 and Laurila and Aho 1997). The oviposition site that the adult anuran selects considerably influences hatching success, larval performance, and in turn affects the parental fitness. Therefore, the ability of the adult anurans to select suitable oviposition sites on the basis of expected larval performance should be strong. This is particularly true when the respective larval stages cannot migrate to better habitat patches without involving high expenditure of energy which in turn increases rate of larval mortality.

In our investigation of oviposition site selection by anurans in East Khasi Hills, each species of anuran was found to select a unique site for oviposition. All the thirteen (13) species observed during the present survey including *Amolops assamensis, Euphtyctis cyanophlyctis, Fejervarya teraiensis, Hyla annectans, Kaloula pulchra, Leptolalax khasiorum, Odorrana livida, Odorrana mawphlangensis, Polypedates himalayensis, Polypedates teraiensis, Rhacophorus bipunctatus, Rhacophorus maximus* and *Xenophrys parva* selected aquatic habitats or a site close to a water body for deposition of the eggs. Some species like *Amolops assamensis, Odorrana livida, Odorrana mawphlangensis, Leptolalax khasiorum and Xenophrys parva selected forest stream for oviposition. While species like Euphlyctis cyanophlyctis, Fejervarya teraiensis, Hyla annectans, Kaloula pulchra, Polypedates himalayensis, Polypedates teraiensis, Rhacophorus bipunctatus* and *Rhacophorus maximus* selected standing water bodies that are permanent or non permanent. The most generalized mode of reproduction which is commonly found in about 80% of the anuran families is the oviposition in standing water (Duellman and Trueb 1986 and Wells 2007).

Polypedates himalayensis, Polypedates teraiensis, Rhacophorus bipunctatus and *Rhacophorus maximus* of the family Rhacophoridae (tree frogs) deposited their eggs in a foam nest on vegetation close to water bodies. *Xenophrys parva* of the family Megophryidae also deposited egg mass attached to rocks or vegetation close to a water body. The deposition of eggs in a foam nest away from the water body may provide protection to the embryos at the early stages from predation (Mohanty-Hejmadi and Dutta 1988). Similarly, Duellman and Trueb (1986) suggested that when eggs are laid away from water in a foamy mass, the tadpoles develop to a pre-metamorphic stage before falling into water which may be an alternative life history strategy of anurans. Hodl (1992), Magnusson and Hero (1991), considered this strategy to facilitate predator avoidance of eggs and early-stage tadpoles, and to reduce the duration of the larval stage by rapid development during the out-of-water phase. A common suggestion is that foam nests protect the eggs from dessication (Salthe and Mecham 1974; Duellman and Trueb 1986; Hodl 1986).

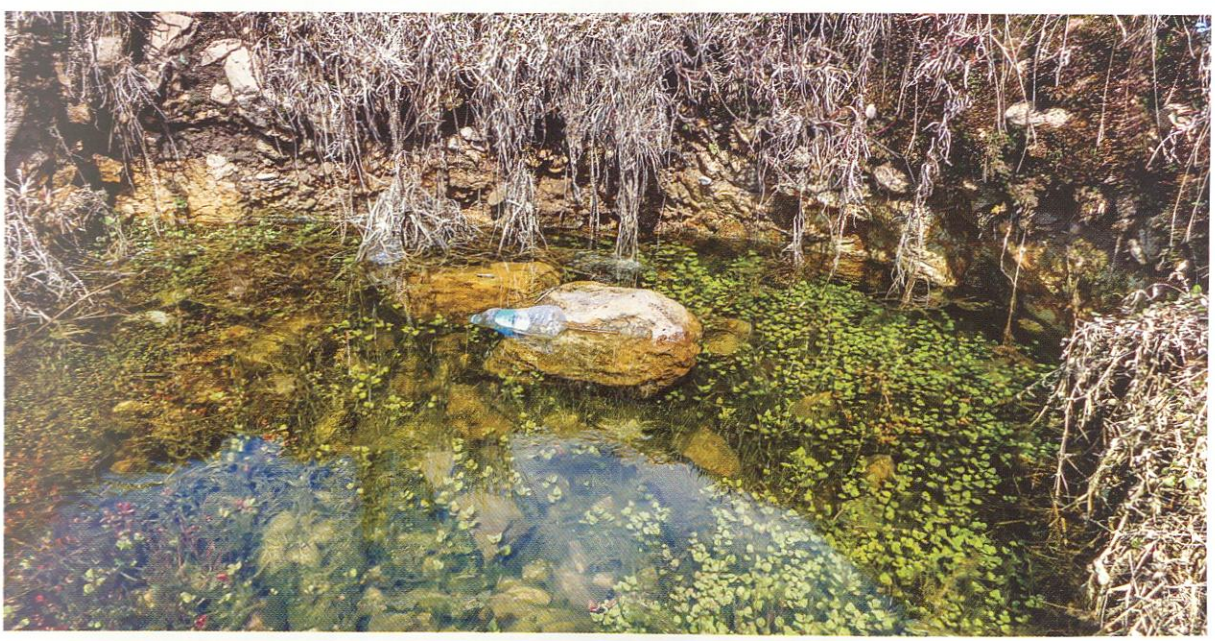
In the present investigation it was observed that egg masses of *Odorrana mawphlangensis* and *Odorrana livida* were similar as in both cases the eggs are non pigmented and are deposited as a clump in water. Aquatic and terrestrial clumps generally forms a multi-tiered stack that lack a common, surrounding surface or

matrix with interstices among eggs and the adjacent jellies remain distinct even if melded (Altig and McDiarmid 2007), as can be seen in the clumps of *Odorrana mawphlangensis* and *Odorrana livida*. Egg masses in *Leptolalax khasiorum, Xenophrys parva* and *Amolops assamensis* also deposited non- pigmented eggs and covered by a transparent jelly. Eggs deposited in hidden locations often are unpigmented as oviposition sites protect the eggs and minimize ultraviolet radiations (Salthe and Mecham 1974). According to various workers, some of the functions of egg jellies include mechanical support for the ovum, attachment of eggs to each other or a structure in the environment (Greven 2002, 2003); enhancement or prevention of entry by conspecific and heterospecific sperm respectively (Barbieri and Del Pino 1970); prevention of polyspermy, sperm capacitation, differential protection from water molds like Saprolegnia and Achlys (Gomez-Mestre et al. 2006); protection from contaminants (Marquis et al. 2006); and protection from predators, pathogens, and environmental stressors such as temperature and UV light (Hunter and Vogel 1986; Itoh et al. 2002; McLaughlin and Humphries 1978; Ward and Sexton 1981). It was observed that *Fejervarya teraiensis, Euphlyctis cyanophlyctis, Kaloula pulchra* deposited pigmented eggs that float on the surface of the water. Eggs that are laid in exposed areas usually have melanic pigment at the animal pole (e.g., *Ambystoma, Bufo, Hyla,* and *Rana* of North America) regardless of the specific site, taxon, or ovipositional mode (Altig and Mc Diarmid 2007). The eggs of *Hyla annectans* were also slightly pigmented and found to be floating on the surface of the water. However, *Hyla annectans* was observed to display a unique behavior in selecting only clean and pristine undisturbed sites for oviposition of the eggs.

In addition to this, it was observed that *Odorrana mawphlangensis, Odorrana livida* and *Amolops assamensis* returned to the same breeding site for oviposition each year. This behaviour is termed as site fidelity, whereby individuals tend to return to a previously occupied site (Bucciarreli 2016). It is presumed that individuals of these species may have site fidelity but mark recapture study was not done in the present investigation. Hence, further studies may be carried out by marking the individuals during the breeding season to prove if they returned back to the same spot for breeding in these specific microhabitats. Thus, implementation of conservation measures of these oviposition sites of amphibians may be considered to be significant. Some amphibians have been reported to display site fidelity to the breeding site, where they often remain in that microhabitat during the entire breeding period (Packer 1963; Crump 1986). Liao (2011) also documented the site fidelity of *Amolops mantzorum* in a montane region in China. Site fidelity may be related to territoriality (Crump 1986) and can be displayed by male frogs that reside for several nights or weeks at the oviposition site to defend against conspecific intruders, which may be a pattern that increases the territorial male's success in attracting females who lay eggs in his territory (Wells 1978); or territoriality displayed by female frogs for sites that can provide high-quality oviposition environment and abundant food resources (Wells 1977b). According to Crump (1986), anurans can also return to the same site due to ideal microhabitat conditions in terms of moisture, abundance of crevices in which to hide and sufficient overhanging vegetation to provide protection from the sun, other than food.

It may be mentioned that parental care was observed, in the form of male remaining at the oviposition site for a few days after oviposition, in some of the anuran species like *Amolops assamensis, Leptolalax khasiorum, Polypedates himalayensis* and *Rhacophorus maximus*. Similar observations in males of Colostethus subpunctatus has also been reported to remain with the egg masses until they hatch (Stebbins & Hendrickson 1959). According to Wells (1977b), male parental care in many anurans is probably an outgrowth of territorial defence of oviposition sites by males which in turn is related to external fertilization of eggs. Hence a male defending a suitable oviposition site might care for eggs already laid while continuing to attract additional females into his territory (Trivers 1972).

During the period of our investigation (2004 to 2015) in East Khasi Hills of Meghalaya, a general trend of degradation of habitats was observed throughout the different surveyed areas. In due course of time, suitable breeding sites were transformed into non suitable breeding or ovisposition sites for anurans. This can be attributed to the large number of disturbances in the areas in the form of clearing of forests for constructions of roads, buildings and for agricultural purposes. This resulted in loss of many breeding habitats that affected the oviposition site selection of many anurans, especially in species that have a strong preference for a site like *Hyla annectans* (that required undisturbed areas). Species that are more adaptive like *Rhacophorus bipunctatus* and *Polypedates himalayensis* were observed to move to other similar suitable sites nearby. Therefore, conservation of breeding habitats becomes very important for anuran species that show a strong preference for the specific oviposition sites. It may be mentioned that the role of the community has been seen to be very important in the conservation process. Anuran species that selected undisturbed areas for oviposition like *Amolops assamensis, Odorrana livida, Odorrana mawphlangensis, Leptolalax khasiorum* and *Xenophrys parva* were all found in community reserved forests. Community forests are protected through prohibition of human activities due to some cultural or religious beliefs, and therefore these forests remain undisturbed. Coincidentally, these forests serve as good habitats for these anuran species and they remain protected. Hence these cultural practices play a major role in the conservation of the amphibian habitats.



Amphibian breeding habitats are often polluted by improper garbage disposal.

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