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RESEARCH ARTICLE

CHOICE OF OVIPOSITION SITE BY NICOBAR FROG, *HYLARANA NICOBARIENSIS* (AMPHIBIA: ANURA)

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ABSTRACT

Observation on the choice of oviposition site of Nicobar frog, *Hylarana nicobariensis* was conducted under both natural and laboratory conditions. It was found that *H. nicobariensis* is the stream breeder, and the breeding season co-incides with winter season (October – February) in Mizoram where the water became shallow and slow flowing, and the peak period of abundance is December when the water become very shallow with the minimum velocity as well as temperature. The eggs are laid in one clutch, which remains submerged and attached to a suitable substratum in water about 5 cm – 10 cm in depth, such as rocks, boulders, pebbles, aquatic plants, dead logs, even among other laid egg clutches, etc., this ensure that the eggs are not washed away by the water currents as there is no parental care in this species. Communal egg masses were observed in the same oviposition site along the rivers or streams and it is suggested that this may confer advantages because the eggs become warmer, the risk of predation is decreased and closely positioned egg masses often adhere to each other as the jelly coat absorbs water or as the water level of the pool decreases. To test the oviposition site choice trials in 23 amplexing pairs in the laboratory, the point bi-serial correlation coefficient was calculated for the successfulness of mating and the temperature under observation for the 11 pairs kept in the terrarium without any substrata. The result revealed highly negative correlation ($r_{pbis} = -.84$) with the statistically probability ($p > .001$). It is also suggested that the availability of substratum with low temperature provided the ambient condition for laying the eggs in the terrarium.

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INTRODUCTION

Most animals lack parental care. Parents choose a suitable environment for oviposition and lay eggs, at which point parental investment is completed and offspring are left to develop on their own. Oviposition site choice is therefore an extremely important decision for parents and selection should act strongly to shape oviposition decisions. Laying eggs in a suboptimal habitat has strong survival costs to offspring and parents should be able to correctly discriminate potential risks to their offspring (Resatarits, 1996). The selection of oviposition sites is an important aspect of reproduction for oviparous species (Snider and Janzen, 2010) as it can affect reproductive success, specifically, embryo survival, hatching success, and larval development (Figiel and Semlitsch, 1995; Rudolf and Rödel, 2004; Snider and Janzen, 2010).

Ideally amphibians should oviposit in areas where abiotic and biotic factors favor the survival and growth of their eggs and larvae (Kats and Sih, 1992; Spieler and Linsenmair, 1997; Rudolf and Rödel, 2004). Factors such as water depth (Petranka and Petranka, 1981; Goldberg *et al.*, 2006; Pearl *et al.*, 2007), water temperature (Doody, 1996; Goldberg *et al.*, 2006; Snider and Janzen, 2010), vegetation (Nyman, 1991; Brodman and Jaskula, 2002; Pearl *et al.*, 2007; Dvůřák and Gvůzdík, 2009), interspecific competitors (Lin *et al.*, 2008; von May *et al.*, 2009), intraspecific competitors (Resatarits and Wilbur, 1989), cannibals (Sadeh *et al.*, 2009), predator abundance (Semlitsch, 1987; Resatarits and Wilbur, 1989; Kats and Sih, 1992), and pollutants (Vonesh and Buck, 2007) can influence oviposition site selection because of their differential effects on survival of offspring. Anuran amphibians can deposit their eggs in a wide range of aquatic habitats. Yet, the diversity of aquatic habitats in which anurans specialize, from temporary storm puddles to large, permanent lakes, or extensive eutrophic river swamps to oligotrophic

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mountain streams, suggest that all water is not equally suitable. A species that typically breeds in temporary ponds is unlikely to oviposit in a rushing stream, or, having done so, be expected to realize the same fitness. Clearly, there is some level of discrimination among sites, at least at that level (Resetarits, 1996). Crump (2000) reported that some anuran species have tadpoles that can develop successfully only in still water, whereas others are adapted to flowing-water systems. Suitable water bodies often are relatively scarce within the landscape, and the attributes likely to influence their suitability for oviposition may include factors, such as availability of vegetated calling sites close to the water's edge (Pough et al., 2004). Oviposition sites are a critical parameter of reproductive success in any species that does not move its young immediately after laying. Therefore, Eterovick and Barros (2003) reported that the influencing role of adults in choosing mating sites limits tadpole distribution among different streams, as distribution of tadpoles and their temporal patterns of occurrence result from the spatial and temporal distribution of reproductive effort by adult frogs, which can be influenced by many factors other than the ecological requirements of their larvae (Alford, 1999). This paper deals with the information on the choice of oviposition site selected by female *Hylarana nicobariensis* during the breeding season and the occurrence of communal egg masses in the breeding habitat.

MATERIALS AND METHODS

Study sites

After surveying different habitats in Mizoram, two study sites designated as study sites I and II were selected from Kolasib district and Aizawl district, respectively. The characteristic features of the study sites are as follows:

Study site I

It is a section of Tuitun stream (23° 58' 21.27" N and 92° 41' 05.51" E; elevations = 300 m – 325 m asl.), Kolasib district (Fig. 1). The river gradient is steep and the width and depth vary considerably, related to rainfall patterns, but on average the river is about 8 m - 15 m wide and 50 cm - 360 cm deep in the center. The river has a permanent flow through alternating riffles, steep waterfalls and pools over bottoms of sand, gravel, huge boulders and bed rock. The river banks consist of sand, mud, gravel, boulders and rock faces. This site is situated about 60 km to the north from Aizawl city. The area is dominated by shrubby vegetation like *Ageratum conyzoides*, *Bidens biternata*, *Crassocephalum crepidioides*, *Osbeckia crinata*, *Eupatorium riparium*, *Colocasia* sps., *Heydichium* sp., *Pterris* sps., *Mussauenda glabra*, *Spilanthes acmella*, *Thysanolaena maxima*, *Chromolaena odorata*, etc., bamboos are *Dendrocalamus* sps., *Neohouzeua dulloa*, *Bambusa tulda*, etc. and trees are *Careya arborea*, *Shorea robusta*, *Tectona grandis*, *Duabanga grandiflora*, *Mesua ferrea*, *Michelia champaca*, *Schima wallichii*, etc. Remaining forest areas are subject to varying levels of human disturbance from selective timber cutting, jhumming cultivation, rattan harvesting and hunting.

Study site II

It lies in the 23° 48' 24.66" N - 55.04" N and 92° 38' 44.51" – 39' 08.97" E along the stretch of Tlawng river, Aizawl district. The altitudes range from 35 m to 50 m asl. It is located about 60 km to the west from Aizawl city. The habitat of this area until fairly recently consisted of mixed semi-evergreen and bamboo forest. Most of this habitat has now been cleared by anthropogenic activities such as teak (*Tectona grandis*) plantation, logging and jhum farming. However some isolated patches still remain.

Study species

Hylarana nicobariensis commonly known as Nicobar frog was encountered in a wide variety of habitats, from running waters and their adjacent water bodies, to rice paddy fields, to leaf-litters of forest floors, disturbed environment with the elevation ranging from 46 m to 1460 m asl in Mizoram (Lalremsanga, 2011). From northeast India, Sen (2004) reported the species from Assam and Tripura only. Outside northeast India, it is also reported from Nicobar Island, Philippines, Thailand, Bali to Peninsular Thailand including Sumatra and Borneo (Frost, 2008). It is a seasonal stream-breeder and the breeding takes place from October to February, sometimes even up to March depending on the timing of monsoon which coincide with late autumn to winter season in Mizoram (Lalremsanga, 2011).

Field surveys

To study the breeding behavior of this species, field surveys in both the study sites were conducted in the evenings from 2:00 PM and continued till late at night during the breeding season. Acoustic Encounter Surveys (AES) are used to identify locations where adult animals are attempting to breed. During the survey period from the year 2004 to till the date, each selected sampling site was covered at different times of the day (from 2:00 PM to 11:00 AM on the following day) in order to record their breeding behaviours.

Oviposition site choice test and statistical analysis

To measure the oviposition site choices of *H. nicobariensis* pairs, oviposition site choice trials were conducted. In this observation, a total of 23 amplexing pairs were captured from the study sites. As the species is a solitary breeder, an amplexus pair was released and allowed to breed in the terrarium (60cm x 30cm x 30cm) filled with water about 5cm depth. Within this course of study, out of 23 amplexing pairs, 12 were kept in the terrarium provided with a boulder at one corner that will serve as a substratum for the newly hatched eggs. Another 11 amplexing pairs were kept in the terrarium without a boulder, but simply with water. Thus, the study incorporated the observation of 12 couples of the amphibians in the presence of substratum and 11 couples in the absence of substratum. The temperature (°C) during each observation is also recorded and the data are processed and analysed with SPSS 21.0.

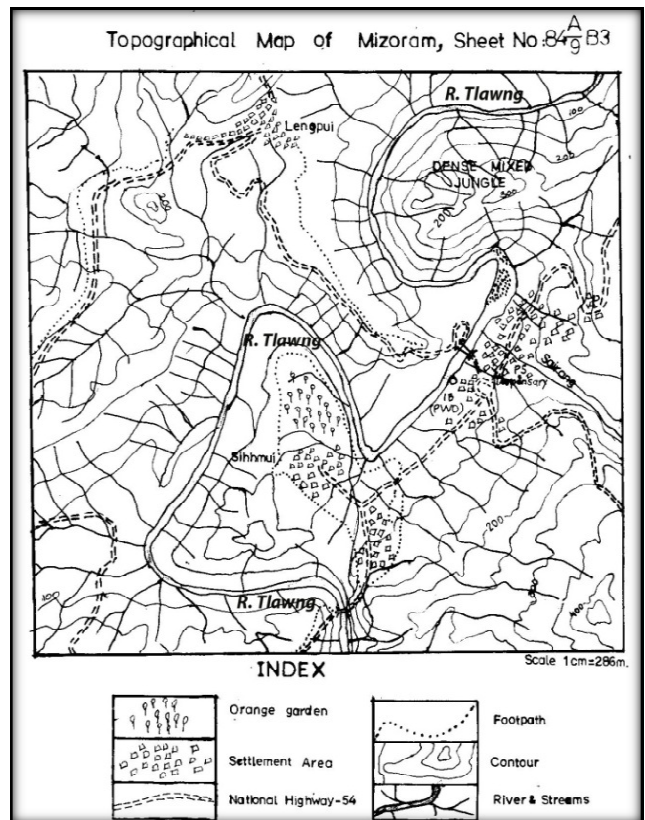
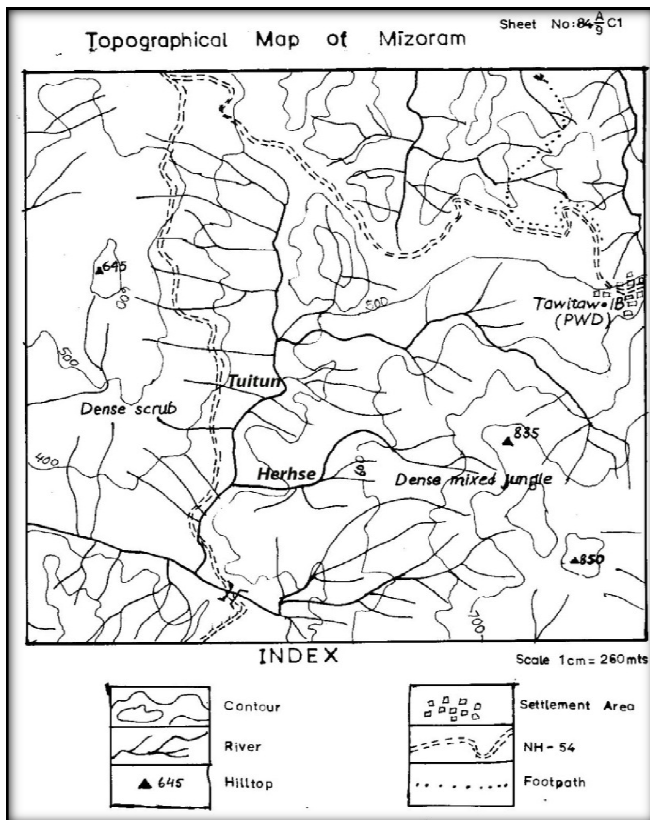
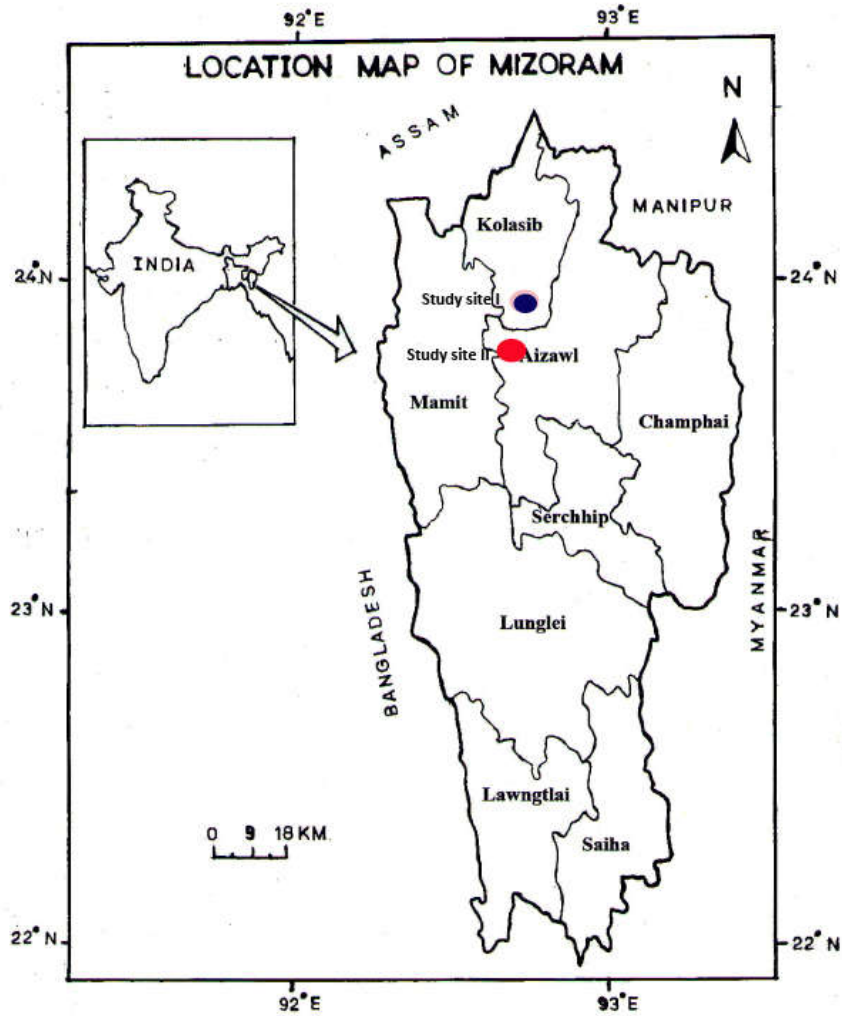


Fig. 1. Map of Mizoram showing study site I and study site II

RESULTS

The natural breeding habitats of *Hylarana nicobariensis* in Mizoram are primarily streams, rivers, ponds and water-filled depressions. However, none of these sites are stable throughout the year, over the course of which, many, particularly stream and river, flood and become largely unsuitable for the species reproduction, especially, during rainy season in Mizoram.

Breeding and Oviposition

Field observations indicated that during the breeding season (October - February), the water became shallow and slow flowing and it was observed to occur within non-rainy season, and the peak period of abundance is December when the water become very shallow with minimum velocity, no rain fall at all, and the ambient air temperature and water temperature between 11.5°C - 29°C and 14.5°C - 19.5°C, respectively. The frogs were observed in cracks of soil and boulders, in pebbles or holes of dead logs, partially submerged in water; few were also located floating on water. Male mating vocalization was heard from cracks of soil and rock near the bank, pebbles and boulders near water, from holes of dead logs, twigs and leaves partially submerged in water, by floating among aquatic plants, or from shallow water. The males call mainly during night beginning at 4:30 PM and unsuccessful males continued until the following day (6:00 AM). In response to the mating call, females came out from their hides among the surrounding vegetations and approached the breeding ground. Within a short period amplexus takes place. The amplexus was axillary and amplexing pairs floats on water or perch on the substrate until eggs are laid (Fig. 2a). However, mating congregation and combat between the males were not seen in this frog; rather, they prefer solitary places for breeding.

Occasionally, amplexing pairs were also encountered among leaf litters even up to 150 m away from the breeding ground. Amplexus (duration: 1 – 6 hours) and egg laying (duration 10 – 20 minutes) occurred both day and night. The eggs are laid in one clutch, which remains submerged and attached to a substratum in water about 5 cm – 10 cm in depth, such as rocks, boulders, pebbles, aquatic plants, dead logs, even among other laid egg clutches, etc. (Fig. 2b - e). The clutches of eggs were usually laid in the sites of pool zone where the velocity of water is less and no oviposition sites were encountered in the riffle zone or fast flowing river or stream water. If the oviposition site was encountered along the torrent section, it was always in the peripheral curved spot where the main rushing water with high velocity did not passed through it (Fig. 2c). The clutch sizes recorded during this period ranged from 131 – 628 (Mean \pm SE= 405.62 \pm 22.75; n=60). No correlation was, however, observed between female body size and clutch size ($r = 0.230$; $p = 0.076$). Distinct sexual size dimorphism was observed in this species where females were larger (Mean \pm SE= 51.56 \pm 0.55; range= 42.36 - 58.6 mm; n=60) than males (Mean \pm SE= 39.06 \pm 0.51; range= 32.34 - 48.48mm; n=60), and males are with darker throat and oval flat gland on the inner arm.

Communal Egg Masses

After laying clutch of eggs, both the male and female move away from the breeding site and the eggs are left to develop on their own (Fig. 3). Sometimes, more than one clutch of eggs (even up to 27 clutches) were seen in the communal egg masses adhered with each other on the substrata in the same oviposition site along the rivers or streams. It was found that the female oviposit eggs in the vicinity or with the existing egg masses of the same species.



Fig. 2. (a) Amplexing pair, and (b – e) different oviposition sites of *H. nicobariensis* in the study sites

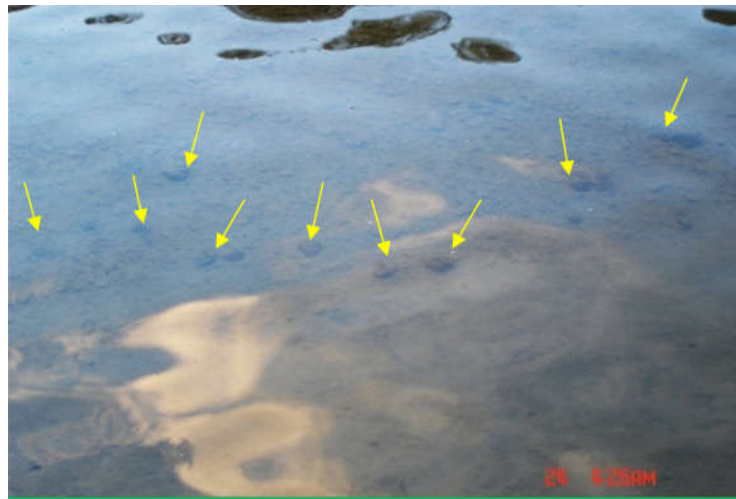


Fig. 3. Group of egg clutches of *Hylarana nicobariensis* in the natural environment

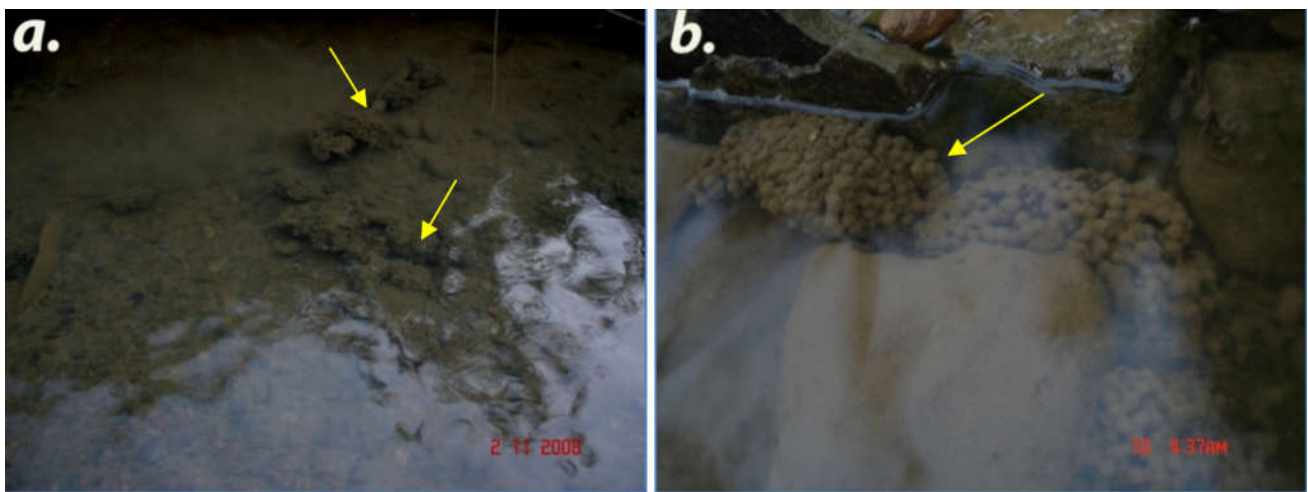


Fig. 4 (a&b). Communal egg masses deposited by females of *Hylarana nicobariensis*



Fig. 5. (a) Amplexing pair and (b): eggs clutch oviposited on the substratum (boulder) in the terrarium

Table 1. The frequency of successfulness of mating with eggs on the substratum, eggs in the water, and no egg for the observation under the substratum present and substratum absent with the mean temperature in °C

Substratum	Egg on the Substratum	Egg in the Water	No Egg	Total	Temperature (°C)
Present	12	0	-	12	13.75°
Absent	-	7	4	11	16.59°
Total	12	7	4	23	15.11°

Communal egg masses were usually encountered among the substrata like, in between rocks and boulders, attached on the dead logs or branches in the pool zone of the stream or river course (Fig. 4a&b). The egg was with animal pole, vary from 1.2 mm – 1.4 mm in diameter. Field observations in the present study revealed that during the non-breeding season as the river was flooded, the frogs move up to the woodland and could only be traced in moist leaf litter in the forested area near the river which was about a hundred meters away from the breeding ground. During the months of March and April although tadpoles, sub-adult and some adult of *Hylarana nicobariensis* were encountered, no sign of breeding behavior was observed and the animal start to retreat in the forest area from the water bodies along the rivers or streams.

Oviposition site choice test and statistical analysis

Result on the observation conducted in the 23 amplexing pairs for oviposition site choice test in the terrarium under laboratory condition clearly revealed successful mating with eggs on the substratum for all the observations (i.e.12 pairs) where in substratum is available (Fig.5a & b). However, only 7 pairs show successful mating with eggs in the water and 4 pairs laying no eggs. In view of the presence of substratum only for the first 12 observations, the point bi-serial correlation coefficient was calculated for the successfulness of mating and the temperature under observation for the later 11 pairs. The result revealed highly negative correlation ($r_{pbis} = -.84$) with the statistically probability ($p > .001$).

DISCUSSIONS

The breeding season of *Hylarana nicobariensis* coincides with winter season (late October - February) in Mizoram which was also observed among other stream breeders e.g. *Hylarana (Rana) longicrus* and *Hylarana (Rana) sauteri* (Kuramoto et al., 1984) from Taiwan, *Hylarana (Rana) sakuraii* from Honshu, Japan (Matsui and Matsui, 1990), *Hylarana (Rana) swinhoana* (Kam, et al., 1998), four species of the *Hylarana (Rana) narina* complex (Kusano and Hayashi, 2002), *Hylarana (Rana) chalconota* and *Hylarana (Rana) celebensis* (Gilliespie et al., 2004). From the present investigation it may be suggested that shallow water and low current during non-rainy season supports survival of larvae, and provides more calling sites and oviposition sites. Moreover, the winter season allows the species to escape from competition with those of monsoon breeders. Up to now, detailed information on the seasonal activity and subtropical regions is lacking; however the available data indicate that the stream breeders reproduce in the dry season (Menzies, 1963; Crump, 1974; Zug and Zug, 1979; Jorgensen et al., 1986; Aichinger, 1987; Gascon, 1991; Kam et al., 1998). However, it was suggested that environmental variables such as humidity, temperature and photoperiod may determine anuran breeding period as reported by earlier workers (Navas, 1996; Navas and Bevier, 2001; Hatano et al., 2002). Observation on the axillary amplexus in *Hylarana nicobariensis* at night time was also reported among other stream breeding frogs e.g. *Hylarana (Rana) longicrus* and *Hylarana (Rana) sauteri* in Taiwan (Kuramoto et al., 1984). An interesting finding is that the breeding peaks of *Hylarana nicobariensis* occurred during December, when the

water become very shallow with minimum velocity, when most frogs were relatively inactive. Kam et al. (1998) suggested that stream breeders may have a different reproductive phenology from ephemeral pond breeders, because water is readily available throughout the larval period and dessication is never a threat to the larvae that live in the water. Fukuyama and Kusano (1992) also reported that floods during wet season reduce the breeding activity of a riparian frog, *Buergeria buergeri*, by reducing the suitable calling sites, such as emerged stones in the flowing riffles. This study also suggested that floods not only reduce suitable calling sites, such as emerged stones in the flowing, but also causes larval mortality and at the same time the low water flowing during the dry season ensure better survival of their offspring as observed by other workers (Metter, 1968; Zug and Zug, 1979; Petranka, 1984; Petranka and Sih, 1986; Kam et al., 1998).

Hylarana nicobariensis deposited egg masses on the substrata (pebbles, rocks, boulders, aquatic plants or wood debris) below the water surface of slow-flowing or still pools in streams and rivers. It may be suggested in the present observation that the eggs are deposited near rocks or boulders, so that the eggs are not washed away by the currents as there is no parental care observed in this species. As it is also reported that the selection of a suitable oviposition site is of critical importance in the reproductive success of organisms that lack parental care (Murphy, 2003). Offspring survival should be dependent on both biotic and abiotic characteristics of the breeding site (Wells, 1977), such as food level, water depth (Crump, 1991), water temperature (Herreid and Kinney, 1967), and the presence of potential predators and competitors (Laurila and Aho, 1997; Resetarits and Wilbur, 1989). Iwai et al., (2007) reported that adult frogs are predicted to select oviposition sites of high quality where these characteristics will be appropriate for their offspring. Several studies indicate that the sites where eggs are oviposited can be influenced by the physical features of breeding sites as well as by the presence of predators, competitors and communal egg masses (Crump 1971, Maiorana 1976; Resetarits and Wilbur, 1989; Donnelly and Guyer, 1994; Holomuzki, 1995; Laurila and Aho, 1997).

During the present investigation, many clutches of egg even up to 27 clutches were seen in the communal egg masses adhered with each other in the same breeding site along the rivers or streams which was also observed among a common brown frog, *Hylarana (Rana) japonica* that breeds during early spring in Japan (Iwai et al., 2007). It is suggested that communal egg masses may confer advantages because the eggs become warmer (Seale, 1982; Waldman, 1982), and the risk of predation is decreased (Håkansson and Loman, 2004). Depositing eggs in sites that have resident eggs may be advantageous for the new eggs in species that potentially form communal egg masses. Moreover, closely positioned egg masses often adhere to each other as the jelly coat absorbs water or as the water level of the pool decreases as it was observed among *Rana japonica* (Iwai et al., 2007). Our results suggest that individuals of *H. nicobariensis* select oviposition sites based on potentially important microhabitat variables. Specifically, we found that *H. nicobariensis* chose to oviposit in areas characterized by substratum (eg. boulder) as well as lower water temperature both in the natural and in the laboratory. The findings of the study under laboratory

condition revealed that: i) availability of substratum is indispensable to avoid unsuccessful mating for the 23 couples under study; ii) lower temperature favours successful mating; and iii) the availability of substratum with low temperature provided the ambient condition of mating for this species.

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