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Breeding site selection of some anurans in Meghalaya in relation to the physicochemical variables of water

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Abstract

Anurans are considered to be the excellent bio-indicators that determined the global health and resilience of the ecosystem. Water temperature, pH, dissolved oxygen and free carbon dioxide may influence the breeding sites of anurans. These factors were analysed to investigate if any of the water physicochemical variables has any effect on the choice of breeding sites by anurans. The investigation was conducted at three breeding sites of anurans in Mawsynram, Meghalaya. Eight anuran species belonging to the family Bufonidae, Dicroglossidae, Rhacophoridae and Hylidae were commonly found to breed in the three breeding sites. It was recorded that 87.5 % of the studied anuran amphibians preferred to breed at study site I (fish pond), whereas 50% and 25% anuran species select study site II and III (rain fed pools) respectively. The water pH was found to differ significantly among the three study sites ($p<0.001$). Dissolved oxygen also differed significantly only between sites I - II and II - III ($p<0.01$), but not between I - III. Water temperature and free carbon dioxide did not show any significant difference in the three study sites. The present investigation revealed that pH and dissolved oxygen of study site I may play an important role in providing a congenial breeding ground to the anuran species prevalent in the area.
The results are discussed with available literatures and suggest that future studies is crucial for the management and conservation of the breeding sites for long term persistence and sustainability of the whole amphibian community.
Key words: anuran, temperature, dissolved oxygen, free carbon dioxide, pH

Introduction

Amphibians form an important component of the food chain as carnivores and prey species in forest and wetland ecosystems and hence constitute a significant proportion of the vertebrate biomass (Burton and Likens, 1975; Gibbons et al., 2006). Intraspecific communications in anurans is mediated by male advertisement call for male-male competition and mate choice (Yoo and Jang, 2012). This is followed by amplexus for successful fertilisation of the egg. Further, climate-specific abiotic factors are primarily linked with anurans reproduction (Bertoluci, 1998; Oseen and Wassersug, 2002; Xavier and Napoli, 2011). Selection of a particular oviposition site associated with amphibian anuran breeding behaviour is of critical importance as it can affect larval development (Resetarits, 1989),

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distribution of tadpoles (Alford, 1999) and the reproductive success of those species with limited parental care (Murphy, 2003). Continuous supply of water is critical for amphibians than most other vertebrate groups as they have dual life cycle and anamniotic egg. Amphibians breed in a variety of aquatic bodies such as streams and springs, temporary ponds, semi permanent ponds. Accumulated water and microhabitats that retain moisture such as burrows, tree holes, rock crevices and fallen logs were utilized by amphibians in seasonal environments and hence have a very short reproductive period (Duelman and Trueb, 1986; Wells, 2007). Hence, for further development of the eggs, most anuran terrestrial breeders depend on water. (Gomez-Mestre et al., 2012).

A critical period in the life history of many amphibian species is their aquatic phase as they displayed a biphasic life cycle with aquatic larvae and terrestrial adults. Shaping of community dynamics and structuring populations depends on habitat selection in mobile organisms (Morris, 2003). Parental fitness, survival of the offspring and population recruitment are largely influenced by selection of breeding-habitat (Resetarits, 1996). Some reports are available on different environmental variables that may have differential effects on the offspring and therefore plays an important role in breeding site selection including 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 (Pearl et al., 2007; Dvorˇ a´k and Gvozˇ d´ık, 2009), interspecific competitors (Lin et al., 2008; von May et al.,2009), intraspecific competitors (Resetarits and Wilbur, 1989), cannibals (Sadeh et al., 2009), predator abundance (Resetarits and Wilbur, 1989), and pollutants (Vonesh and Buck, 2007).

Several physicochemical properties of the water have an important role in aquatic life stages of anurans. Those that affect survival, growth, maturation and physical development are dissolved oxygen, water temperature, pH, salinity, organic carbons and water conductivity (Sparling, 2010). Retardation of development and death occurs if the eggs are laid deep in the water column, where oxygen is low. Aquatic eggs in some anurans such as *Hypsiboas rosenbergi*, suffocate if they fall to the pond bottom (Touchon and Warkentin, 2008). There are intraspecific variations among amphibians towards acid tolerance and some are sensitive even at low levels of acidity while few others can survive relatively low pH conditions, with minor effects on growth and developmental processes (Pierce, 1985; Devi et. al., 2016). Studies also reported that for larval growth and habitat selection, temperature is a critical factor (Goldberg et al., 2009). Therefore to maximise the individual fitness and for the long term persistence of the whole amphibian community, selection of the suitable aquatic bodies for their breeding and further development till the completion of metamorphosis is crucial.

Loss of global freshwater aquatic ecosystem services is indicated by amphibian decline (Lannoo, 2008; Collins and Crump, 2009). Confronting amphibian decline required examining and evaluating the associated ecosystem services (Hocking and Babbit, 2014). In context to the study area of the current investigation (Meghalaya a North East Indian State), it may be noted that there is very little information on the same (Tron et al., 2015; Khongwir et al., 2016). Literature surveys yet revealed scanty information on the effects of the

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physicochemical properties of aquatic body with special reference to the choice of breeding sites by anurans in the region. Hence, it seems essential to gather information on such aspect by identifying their breeding habitats and oviposition sites to understand how these factors affect habitat suitability for different anuran species. This will help to prioritize and support conservative measures that further highlight certain predictions of environmental variable fluctuations associated with the potential loss of the species. This is important as amphibian population decline at a local scale might be due to the poor quality of the water at the breeding sites in combination with other environmental factors.

Materials and Methods

Field surveys were made from three aquatic breeding habitat of anurans during the breeding period from morning 08.00 to 23.00 h for two consecutive years 2017 to 2018 throughout the different parts of Mawsynram area (25° 28′ N, 91° 35′ E) at an altitude of 1300 to 1400 m asl., a sector on the southern slopes of Meghalaya, North East India. During field surveys three study sites were selected for the study (Figure 1). It was noted that Study site I is a temporary rain fed water body used earlier as a fish pond, measuring 72 m2 and is surrounded by vegetation mainly grasses such as *Cynodon dactylon* and *Erianthus fulvus*. Study site II is a temporary rain fed pond measuring about 46 m2 and surrounded by grass *Erianthus fulvus*, shrubs *Quercus glauc*a and trees *Myrica esculenta*. It is used as a dumping site by the local people. Study site III is also a temporary rain fed pool situated at the base of the limestone mine measuring approximately 17 m2 and has less vegetation cover surrounded mainly by grass *Cyanodon dactylon* and shrubs *Castonopsis indica*. Standard survey techniques such as Visual Encounter Survey were followed for adult amphibians. During night time, study plots were surveyed with the help of head lamps and torch lights. During surveys the microhabitat and the breeding sites of anurans for oviposition were noted. The breeding activities of the observed anuran species were photo documented at the study site using Nikon D3400. Few specimens from the study sites were collected and brought to the laboratory for making careful observations and morphometric measurements were carried out using a dial caliper (Mitutoyo series No. 505-671), few were sacrificed by anesthetization with Tricane methane Sulfonate- MS 222 solution and then fixed in 4 % formaldehyde for preservation for future studies where as others were released back into the natural habitat. The preserved specimens were identified with the help of the monograph key prepared by Chanda (1994) and with the help of Zoological Survey of India, Shillong and also with the help of available literatures. During surveys, the water temperature was recorded and the dissolved oxygen, free carbon dioxide and pH of the aquatic habitat were recorded at weekly intervals and analysed following the standard protocols (A.P.H.A, 1992). Data were collected between 18.00 h to 23.00 h when anuran activity was maximum. The difference in the water physicochemical variables among the three study sites were statistically analysed with Graph Pad Prism 4 (www.graphpad.com) using the Bonferonni test.

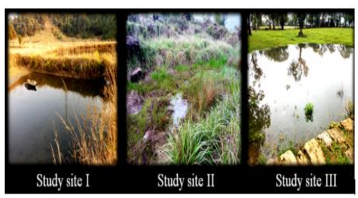

Figure 1: The three selected study sites in different parts of Mawsynram area (25° 28′ N, 91° 35′ E), Meghalaya, North East India. Study site I, II and III are rain fed ponds.

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RESULTS

From the study conducted, eight anuran species (*Duttaphrynus melanostictus, Fejervarya nepalensis, Hyla annectans Polypedates himalayensis, Polypedates teraiensis, Rhacophorus maximus Rhacophorus bipunctatus, and Kurixalus naso*) belonging to 4 families (Bufonidae, Dicroglossidae, Hylidae and Rhacophoridea) were observed to select the three different water bodies (Study site I, II & III) for their breeding activity. Further, it was observed that the breeding site preference for the three study sites by the anuran species differs considerably (Table 1). Out of the eight anuran species recorded, *Duttaphrynus melanostictus* and *Rhacophorus maximus* were found to breed in all the three study sites. *Fejervarya nepalensis, Hyla annectans, Polypedates himalayensis*, and *Kurixalus naso* breed only at study site I. *Rhacophorus bipunctatus* select site I and II for its breeding activity. However, Polypedates teraiensis was recorded only at study site II. It revealed that 87.5 % of the studied anuran amphibians were found to breed at study site I, whereas 50% and 25% anuran species choose study site II and III respectively.

Table 1: Anurans species recorded in the three breeding sites (I, II & III).
'+' sign indicates presence of breeding activity of a species at the study site.
'-' sign indicates absence of breeding activity of a species at the study site.

Sl no.	Family	Species	Study site I	Study site II	Study site III
1	Bufonidae	*Duttaphrynus melanostictus*	+	+	+
2	Dicroglossidae	*Fejervarya nepalensis*	+	-	-
3	Hylidae	*Hyla annectans*	+	-	-
4	Rhacophoridae	*Polypedates himalayensis*	+	-	-
5	Rhacophoridae	*Polypedates teraiensis*	-	+	-
6	Rhacophoridae	*Rhacophorus maximus*	+	+	+
7	Rhacophoridae	*Rhacophorus bipunctatus*	+	+	-
8	Rhacophoridae	*Kurixalus naso*	+	-	-

The onset and duration of breeding activity differs among the eight species of the anurans observed in the three selected study sites at an altitude of 1300 to 1400 m asl. *Duttaphrynus melanostictus* is a seasonal breeder whose breeding activity coincides with the onset of few showers of rainfall i.e. from March to April. *Fejervarya nepalensis* breeds throughout the months of May to July while *Hyla annectans* was found to breed during the early part of the monsoon season from April till May. The present study indicated that there was a variation in the onset and duration of breeding activity among the 5 rhacophorid species. The breeding activity of *Polypedates himalayensis* was recorded from March to June, while *Polypedates teraiensis* was found to breed from the month of March to early July. It was observed that *Rhacophorus maximus* begins its breeding phase with the first shower during the month of March and it last till early May. The species *Kurixalus naso* however starts its breeding activity during the month of February and it last till March. The breeding period of *Rhacophorus bipunctatus* was observed from April to July.

The present investigation revealed that among the five rhacophorids, *Polypedates himalayensis* and *Polypedates teraiensis* oviposited in foam nests constructed at the grassland vegetation close to these water bodies of site I and site II respectively. Further, the present observations on the oviposition of *Rhacophorus maximus* showed that they construct foam nests on any substrata where water is available in all the three study sites. However, *Rhacophorus bipunctatus* oviposited on the leafy vegetation and rotten logs above the water surface in site I & II. It was also found during surveys that *Kurixalus naso* oviposited in the moist soil at study site I. The aforementioned findings revealed that the rhacophorids selected aquatic habitats and sites which are close to water bodies. However, rainfall appears to play a critical role in transporting the eggs deposited at the sites other than water

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(vegetation, burrows), in to the nearby water bodies for further development of the larvae and completion of metamorphosis. *Duttaphrynus melanostictus, Fejervarya nepalensis* and *Hyla annectans* oviposited in the water and hence shows aquatic oviposition. It was also observed that study site II which supports the breeding of 4 anuran species was used as a dumping site by the local people.

The water physicochemical parameters recorded at weekly interval for two consecutive years (2017-2018) showed that all the three sites are shallow temporary rain fed pools which remain dry from the month of August to February. Water accumulation was observed at the three study sites with the appearance of the monsoon that initiate the breeding of the anuran species. Interestingly it may be mentioned that in the year 2018 the breeding sites have a relatively short hydro-period (March-June). Study site I revealed that the mean temperature of the water ranged from 14.40ºC to 25.27 ºC, average dissolved oxygen and free carbon dioxide ranged from 3.22 mg/l to 8.10 mg/l and 3.38 mg/l to 9.52 mg/l respectively (Table 2a, 2b &2c). However, pH was recorded to range from 5.11-6.89 (Table 2d). In the study site II, the mean water temperature ranged from 13.30 ºC to 22.10 ºC, average dissolved oxygen and free carbon dioxide ranged from 4.15mg/ml to 9.62 mg/l and 6.54 mg/ml to 9.75 mg/l respectively (Table 2a, 2b &2c), where as the mean pH range was found to be highly acidic (4.01 to 5.60) (Table 2d). In study site III, the mean water temperature ranged from 14.50 ºC to 22.67 ºC, dissolved oxygen 3.91mg/l to 6.24 mg/l and free carbon dioxide ranged from 6.28 mg/l to 9.66 mg/l (Table 2a, 2b &2c), and the mean pH was found to be above neutral and more towards basicity with a range from 7.22 to 9.33 (Table 2d). It was noted that the three parameters (water temperature, dissolved oxygen and free carbon dioxide) vary in different months in all the study sites (Figure 2a-c). It appeared that with the increase in water temperature, there is a decrease of dissolved oxygen. This follows the general water chemistry as reported by Benson and Krause (1980). Further, the graphical representation (Figure 2a-c) depicted an inverse relationship between free carbon dioxide and dissolved oxygen (Ruttner, 1963). However, pH seems to be an independent variable. When comparing the differences of each water physicochemical variable among the three study sites through the Bonferonni test in Graph Pad Prism 4 (www.graphpad.com), it revealed that Water temperature and free carbon dioxide did not show any significant difference in the three study sites (Figure 3a, 3c). Dissolved oxygen also differed significantly only between sites I - II and II - III ($p<0.01$), but not between I – III (Figure 3b). From the analyses it was observed that the water pH differed significantly ($p<0.001$) among the three study sites (Figure 3d).

Table 2b: Dissolved oxygen (mg/l) of the three selected study sites recorded during the breeding season. All values are given Mean ± SD

Year	Months	Dissolved oxygen (mg/l) ±SD		
		Study site I	Study site III	Study site III
2017	March	8.1±0.08	9.62±0.3	5.54±0.04
	April	6.34±0.04	8.73±0.1	4.95±2.38
	May	3.4±0.2	6.82±0.023	4.04±0.04
	June	3.44±0.08	6.06±0.06	3.91±0.04
	July	4.22±0.22	4.15±0.15	4.58±0.608
2018	March	6.35±0.03	8.52±0.1	6.24±1
	April	4.63±0.03	7.36±0.01	4.58±0.08
	May	3.35±0.15	6.29±0.1	5.186667±0.6
	June	3.22±0.02	9.35±0.25	4.43±0.03
Range		3.22-8.1	4.15-9.62	3.91-6.24
Mean± SD		4.78±1.75	7.43±1.79	4.83±0.74

Table 2a: The water temperature (ºC) of the three selected study sites recorded during the

Year	Months	Mean water temperature (ºC) ± SD		
		Study site I	Study site II	Study site III
2017	March	14.40± 0.098	13.30± 0.3	16.67± 0.02
	April	15.30± 0.6	19.23± 0.01	14.50± 0.05
	May	17.70± 0.061	18.40± 2	18.57± 0.07
	June	21.50± 2	17.30± 0.03	22.40± 0.4
	July	22.50± 4	20.33± 0.33	20.30± 0.01
2018	March	22.43± 0.015	19.30± 0.1	19.57± 0.065
	April	24.40± 0.4	21.40± 0.4	21.73± 0.01
	May	23.43± 0.1	22.10± 0.01	20.16± 0.995
	June	25.27± 0.02	20.43± 0.01	22.67± 0.03
Range		14.40-25.27	13.30-22.10	14.50-22.67
Mean ± SD		20.76±3.99	19.09±2.62	19.55±2.61

breeding season. All values are given Mean ± SD.

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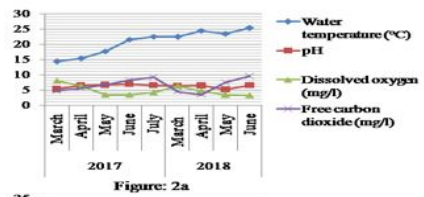
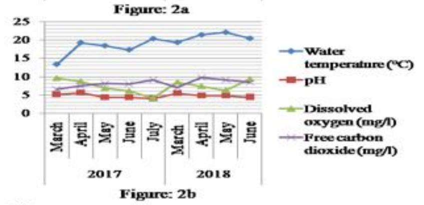
Table 2c: Free carbon dioxide (mg/l) of the three selected study sites recorded during the breeding season. All values are given Mean ± SD.

Year	Months	Free carbon dioxide (mg/l) ±SD		
		Study site I	Study site II	Study site III
2017	March	4.68 ±0.1	6.54±0.54	7.28±0.01
	April	5.51±0.51	7.45±0.05	6.83±0.03
	May	6.58±0.03	8.1±0.1	8.25±0.1
	June	8.25±0.25	8±0.02	9.3±0.3
	July	9.08±0.08	9.12±0.12	8.24±0.04
2018	March	4.41±0.41	7.07±0.07	6.28±0.28
	April	3.38±0.38	9.75±0.05	7.67±0.077
	May	7.34±0.34	9.11±0.11	9.36±0.01
	June	9.52±0.52	8.4±0.4	9.66±0.06
Range		3.38-9.52	6.54-9.75	6.28-9.66
Mean± SD		6.53±2.18	8.17±1.046	8.096±1.18

Table 2d: The water pH of the three selected study sites recorded during the breeding season. All values are given Mean ± SD.|

Year	Months	Mean pH ± SD		
		Study site I	Study site II	Study site III
2017	March	5.22±0.04	5.15±0.025	8.09±0.049
	April	6.53±0.025	5.60±0.3	8.04±0.46
	May	6.63±0.086	4.27±0.3	7.42±0.015
	June	6.89±0.047	4.27±0.017	8.26±0.032
	July	6.54±0.030	4.01±0.04	8.00±0.11
2018	March	6.34±0.032	5.43±0.06	8.01±0.05
	April	6.43±0.049	4.76±0.025	7.22±0.045
	May	5.11±0.032	4.89±0.11	8.76±0.1
	June	6.49±0.090	4.40±0.53	9.33±0.05
Range		5.11-6.89	4.01-5.60	7.22-9.33
Mean± SD		6.24±0.63	4.75±0.56	8.13±0.63





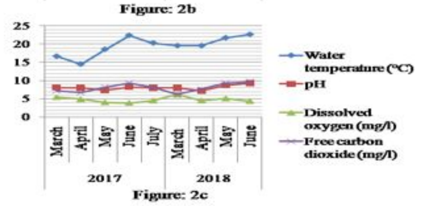


Figure 2a: Average water temperature, pH, dissolved oxygen and free carbon dioxide of Study site I during the breeding period of the eight anuran species (2017-2018)

Figure 2b: Average water temperature, pH, dissolved oxygen and free carbon dioxide of Study site II during the breeding period of the eight anuran species (2017-2018)

Figure 2c: Average water temperature, pH, dissolved oxygen and free carbon dioxide of Study site III during the breeding period of the eight anuran species (2017-2018)

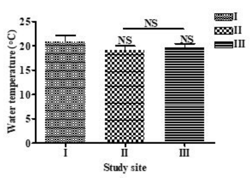


*Figure 3a: The water temperature (°C) of the three selected study sites I, II & III. All values are expressed as mean ± SD; N=9. Asterisks represent different levels of significance as compared with the values of Study site I: *p<0.05, **p<0.01 and ***p<0.001, NS-Not significant at p>0.05 respectively.*

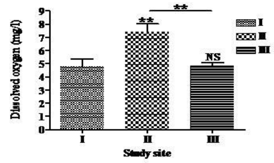
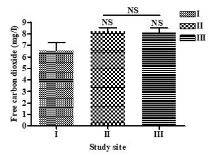


Figure 3b: Dissolved oxygen (mg/l) of the three selected study sites I, II & III. All values are expressed as mean ± SD; N=9. Asterisks represent different levels of significance as compared with the values of Study site I: *p<0.05, **p<0.01 and ***p<0.001, NS-Not significant at p>0.05 respectively.



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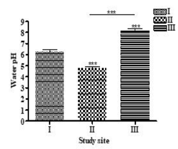

*Figure 3d: The water pH of the three selected study sites I, II & III. All values are expressed as mean ± SD; N=9. Asterisks represent different levels of significance as compared with the values of Study site I: *p<0.05, **p<0.01 and ***p<0.001, NS-Not significant at p>0.05 respectively.*

DISCUSSION

The present investigation indicated that the breeding site selection by anurans was influenced mainly by water pH which differs significantly among the three study sites. Earlier reports revealed that some anurans such as Rana virgatipes and *Hyla andersoni* (Gosner and Black, 1957), *Rana sylvatica* (Freda and Dunson, 1986), *Rana arvalis* (Andren et al., 1988), *Hyla versicolor* (Grant and Litcht, 1993) have the capability to survive under naturally acidic waters. Acidic tolerance of the anuran species might be due to genetic adaptation to acidic environments (Tome and Pough, 1982; Karns, 1983). Similar observations were made on the present study for the four anuran species, *Duttaphrynus*

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melanostictus, Polypedates teraiensis, Rhacophorus maximus and *Rhacophorus bipunctatus* which showed tolerance towards high acidity and adapted to breed at study site II (highly acidic) for their breeding activity. It may be suggested that the source of acidification at study site I and II might be acid precipitation (Haines, 1981) and presence of sphagnum moss at the pond bottom (Gosner and Black, 1957). On the basis of the observations made in the current study, it may be further assumed in this investigation that dumping of some used bottles, plastics and waste matter at study site II might have enhanced the acidity of the water body as suggested by Pierce (1985).

Earlier studies found that at pH 4 some tadpoles showed sluggish movement and reduced feeding rates (Rosenberg and Pierce, 1995). Some authors have reported that at pH 4.0-5.0, hatching percentage is reduced remarkably (Gosner and Black, 1957; Dunson and Connel, 1982). Low pH results in reduced growth, prolonged metamorphosis (Sparling, 2010) and also adverse effects on the immune systems (Brodkin et al., 2003). In addition, other effects of low pH includes reduction in sperm motility and fertilization success (Schlichter, 1981), shrinkage and mechanical constriction of the egg membrane that inhibited hatching (Gosner and Black, 1957; Pough and Wilson, 1977), deactivation of hatching enzyme (Dunson and Connel, 1982) and direct killing of tadpoles (Saber and Dunson, 1978). The aforementioned findings might be the possible causes for failure of the four anuran species *Fejervarya nepalensis, Hyla annectans, Polypedates himalayensis,* and *Kurixalus naso* to breed at study site II in the current study, which is highly acidic (4.01-5.60). However, they can adapt to slightly acidic pH (5.11-6.89) and hence select study site I for their breeding activity.

Further, it may be suggested that basicity of study site III (7.22-9.33) obstructed the breeding activity of most anuran species prevalent in the study area where only *Duttaphrynus melanostictus* and *Rhacophorus maximus* have the ability to tolerate basic pH and hence select study site III. Thus, *Duttaphrynus melanostictus* and *Rhacophorus maximus* have a broader range of tolerance towards pH levels. It may be assumed that water that springs up from the base of limestone mine, entering through small channels at site III during the rainy season might be responsible for the basicity of the water body at study site III. Odum and Zippel (2011) mentioned that majority of amphibians favour slightly basic waters although there is variation among species towards adaptation to different pH level. However, detrimental effects on frogs embryos have been reported at high pH levels (8.10-10.04) in combination with low dissolved oxygen and high water temperatures (Boyer and Grue, 1995). Interestingly, in the present investigation high pH appeared to inhibit the breeding site preference by the six anuran species *Fejervarya nepalensis, Hyla annectans, Polypedates himalayensis, Polypedates teraiensis, Rhacophorus bipunctatus* and *Kurixalus naso* at study site III. It may be suggested that besides water pH a complex array of other factors such as absence of suitable vegetation cover which provides shelter and calling sites for male anurans to initiate the breeding activity and frequency of human disturbances might be responsible for low preference of anurans to choose and oviposit at study site III.

Relatively less information regarding the minimum levels of dissolved oxygen required to maintain the normal physiology for amphibian species is

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available. However, most of the work on this aspect is available in the existing literature for fish species (Odum and Zippel, 2011). It is apparent from the present investigation that the concentration of dissolved oxygen did not affect the choice of breeding site by anurans. However, dissolved oxygen play a critical role for further developmental processes of the anuran aquatic life stages (Sparling, 2010), physiology (Rose et al., 1971) and behaviour (Wassersug and Seibert, 1975). Wells (2007) reported that there is a declining metabolic rate of the anuran larvae during early developmental processes in warm ponds with hypoxic conditions. However, under hypoxic conditions, the anuran tadpoles are adapted through aerial respiration which may account up to 100% of the total oxygen consumption (Feder and Wassersug, 1984) and this is achieved through development of functional lungs in anuran tadpoles prior to metamorphosis (Duelman and Trueb, 1986). On the basis of our observation, it may be suggested that while choosing the suitable oviposition sites, anurans have different adaptive features to meet the oxygen demands of the embryos in the selected study sites. Some reports suggested that eggs are sometimes deposited as surface film and it might be one of the adaptive features to meet the oxygen demands of the anuran embryos (Moore, 1940). This might be true for *Fejervarya nepalensis, Hyla annectans* and *Duttaphrynus melanostictus* where eggs are deposited as surface film. It was reported that for terrestrial gelatinous eggs, little air spaces between the mass may limit oxygen diffusion and becomes fatal to anuran embryos. Further, the oxygen delivery constraints is limited for most terrestrial eggs as they are embedded in foam nest in which most oxygen is supplied by the surrounded foam bubbles (Seymour, 1999). Hence, ambient oxygen might play a role in oxygen diffusion to the embryo for rhacophorid species like *Rhacophorus maximus, Rhacophorus bipunctatus, Polypedates teraiensis, Polypedates himalayensis* and *Kurixalus naso* to adapt to terrestrial and semi aquatic oviposition.

To summarise this present investigation, it may be concluded that the major factor affecting the breeding site selection of anurans are the suitable ecological conditions for larval development, metamorphosis and parental fitness. Wells (2007) reported that the animal chooses a moderate temperature ranging from 15 ºC to 25 ºC for different activities. Similar report was made by Duelman and Trueb (1986) who mentioned that most anuran embryos developed normally within a temperature range of 15 ºC to 20 ºC. These findings support the observation made in the present study in supporting on the breeding activity of anurans in normal water temperature range (13.30 ºC to 25.27 ºC). However, no apparent differences in water temperature and free carbon dioxide were observed at the three study sites, although it is pertinent to mention that understanding the relations between water physicochemical parameters in the field with reference to amphibians is very critical for their conservation. In addition to the present investigation, other multiple biotic and abiotic factors may interact simultaneously for breeding site selection of the eight anurans. However, scanty information is available on the assessment of such multiple factors which may risk the breeding site choices by anurans (Lin et al., 2008). As amphibians are experiencing major declines (Hocking and Babbit, 2014) since last decade, appropriate measures are to be taken for ensuring their conservation. Some of the

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important steps in this regard are: i) restoration of temporary ponds with diverse array of hydroperiods, ii) minimizing the excessive use of pesticides and fertilizers in the agricultural fields adjacent to the anuran breeding sites iii) to avoid utilization of amphibian breeding site as dumping site, iv) maintenance of the vegetation cover to protect the core breeding sites.

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