



## Observation on the breeding biology of *Polypedates teraiensis* (Dubois, 1987) (Amphibia: Rhacophoridae)

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The breeding biology of tree frog, *Polypedates teraiensis* was studied during the breeding season at Mizoram University Campus. It was found that sound production by male during the breeding season was primarily a reproductive function and advertisement calls attract females to the breeding areas and announce other males that a given territory is occupied. The aim of this study was to provide the detailed information on the breeding behaviour and the advertisement calls of *Polypedates teraiensis*. The morphometric measurements of the amplexing pairs (males and females) for sexual dimorphism along with clutch sizes were also studied.

**Key words:** *Polypedates teraiensis*, advertisement calls, breeding, Mizoram, northeast India.

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### Introduction

The social behaviours of most anurans are associated with acoustic communication in the form of vocalization.<sup>1</sup> Advertisement calls are species-specific. The intra-specific diversity in call characteristics allows females to discriminate among potential conspecifics mates based on some of the same acoustic parameters used for species identification.<sup>2</sup> Although most anurans are normally solitary, they often come together in large breeding aggregations during the spring or rainy season. Bioacoustic studies play fundamental roles in understanding and resolving several issues related to the study of anuran amphibians (frogs and toads). Given the importance of acoustic signalling in the breeding ecology of most frogs,<sup>3-4</sup> detailed acoustical and statistical

descriptions of signals are an important first step toward understanding the reproductive and social behaviours of anurans.<sup>5-7</sup> Furthermore, basic knowledge of a species' acoustic behaviour has important implications for conservation in at least two respects. On the one hand, bioacoustic data can be used as a non-invasive tool for the purposes of population census and monitoring. The integration of bioacoustics with other data sources can be important for effective conservation assessment, planning, and management, especially for threatened and endangered species.<sup>8-13</sup> Given the global decline in amphibians,<sup>14</sup> integrating bioacoustics with anuran conservation is an important goal. Quantitative descriptions of animal vocalizations can inform an understanding of their evolutionary functions, the mechanisms for their production and perception,

and their potential utility in taxonomy, population monitoring, and conservation.<sup>15</sup>

The perching frog, six-lined tree frog, or Terai tree frog (*Polypedates teraiensis*) is a species of frog in the Rhacophoridae family. It is found in eastern Nepal, eastern, peninsular, and north-eastern India (West Bengal, Meghalaya, Mizoram, Assam, Arunachal Pradesh, Nagaland, Manipur, Sikkim, also reported for Gujarat and Madhya Pradesh) and Bangladesh, into adjacent Myanmar, and possibly into adjacent China.<sup>16</sup>

## Materials and Methods

### Study sites

During the study period, it was observed that *Polypedates teraiensis* was a seasonal breeder and its breeding activity coincides with the onset of monsoon, i.e. March to August in Mizoram. The study was conducted in the year 2015 and 2016 at two study sites, i.e. Site I: an artificial Pond (circumference = 26.38 m) located near Lianchhiari road (with a GPS location of N 23° 44'15.7": E 92°40'02.5" at an elevation of 824 m asl) and Site II: an old water storage tank (3.54 m x 2.32 m x 2 m) located near Lengteng Boys Hostel (with a GPS location of N 23°44'18.0": E 92° 39'43.2" at an elevation of 775 m asl) inside the campus of Mizoram University, Tanhril, Aizawl. Breeding behaviour, amplexing pairs and foam nests construction were studied and documented with the help of photographic and video cameras.

### Abbreviations used are as follows

**SVL:** Snout-vent length.

**Head:-** **HW:** Head width, **HL:** Head length, **MN:** Distance from the back of mandible to the nostril, **MFE:** Distant from the back of the mandible to the front of the eye, **MBE:** Distant from the back of the mandible to the back of the eye, **IFE:** Distant between the front of the eye, **IBE:** Distant between the back of the eye, **IN:** Inter nasal space, **EN:** Eye to nostril (distance from the

front of the eye to the nostril), **EL:** Eye length, **SL:** Snout length (distance from the front of the eye to the tip of the snout), **SN:** Snout to nostril (distance from the nostril to the tip of snout), **TYD:** Greatest tympanum diameter, **TYE:** Distance from tympanum to the back of eye, **IUE:** Minimum distant between upper eyelids, **UEW:** Maximum width of inter upper eyelids.

**Hind limbs:-** **FLL:** Fore limb length (from proximal end of arm with to tip of longest finger), **HAL:** Hand length (from the base of outer palmar tubercle to tip of finger), **TFL:** Third finger length, **PA:** Width of pads of fingers, **WA:** Width of fingers.

**Hind limbs:-** **FL:** Femur length, **TL:** Tibia length, **TFOL:** Length of tarsus and foot, **FOL:** Foot length, **FTL:** Fourth toe length, **PP:** Width of pads of toes, **WP:** Width of toes, **IMT:** Length of inner metatarsal tubercle, **ITL:** Inner toe length, **T1 :** From base of foot to tip of longest toe, **T2:** From base of foot to tip of second toe, **T3:** From base of foot to tip of third toe, **T4:** From base of foot to tip of fourth toe, **T5:** From base of foot to tip of fifth toe.

**Webbing:-** **MTTF:** Distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between third and fourth toe, **TFTF:** Distance from the maximum incurvation of the web between third and fourth toe to the tip of fourth toe, **MTFF:** Distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between fourth and fifth toe, **FTFF:** Distance from the maximum incurvation of the web between fourth and fifth toe to the tip of fourth toe, **WTF:** Webbing between third and fourth toe (from the base of the first subarticular tubercle), **WFF:** Webbing between fourth and fifth toe (from the base of the first subarticular tubercle).

### Acoustic analysis

Mating calls were recorded with the help of digital voice recorder Sony ICD-PX440 Professional compact voice recorder. The sampling used to convert the signals to digital format was 8 KHz with 16-bit precision. The oscillogram was



Figure 2 | Axillary amplexing in *Polypedates teraiensis* at study site I.



Figure 3 | Amplexing pair constructing foam nest at study site II.

prepared and analysed with the help of a software tool "SoundRuler Version 0.9.6.0 (acoustic analysis)". The notes are composed of groups of pulses. Notes are measured from the beginning of the first pulse to the end of the last pulse; intervals between two subsequent notes are measured from the end of the last pulse of the first note to the beginning of the first pulse of the following note; note repetition rate is the number of notes per second; pulse repetition rate is the number of pulses per second. The data were analyzed with the help of statistical software tools SPSS (7.5.1 version) and OriginPro 8 SRO (8.0724 version).

## Results

### *Breeding season*

In the present observation, the breeding activity of *Polypedates teraiensis* was observed from March where the onset of monsoon had already started. From March to August the frogs came out to mate in the pools. The atmospheric temperature recorded ranges between 26°C to 38°C, water temperature between 24°C to 28°C, pH between 5.54-8.22 during the investigation period in both the study sites.

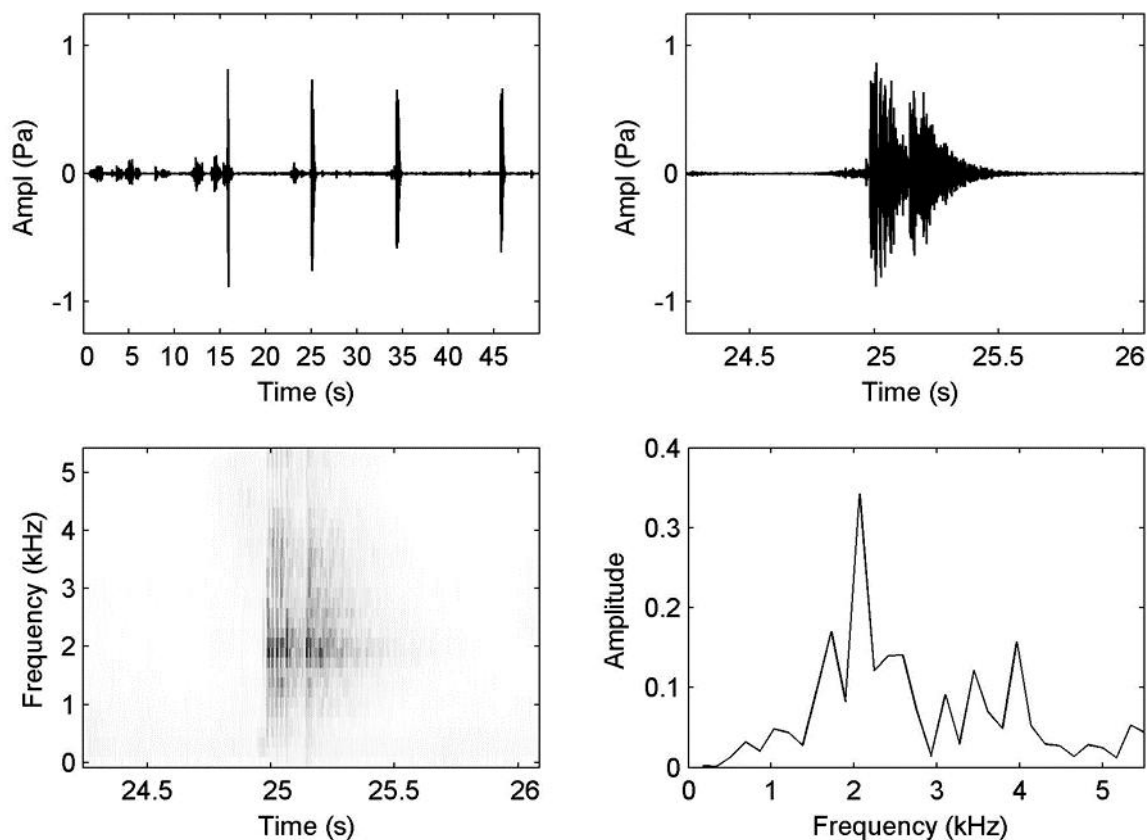


Figure 1 | Oscillogram, sonogram and frequency spectrum of an advertisement call of *Polypedates teraiensis*.

### *Courtship and advertisement calls*

During the study period, adult male frogs were observed to be the first to emerge from their hiding places during the evening and make advertisement calls. It was also observed that multiple males aggregate and produce advertisement calls while hiding behind the grasses, some damp places near the breeding site, or while floating on the water surface. Advertisement calls were audible to the human ear from a distance of 30–35 m. The calling sound was usually heard during the evening and continued till early in the morning. It was observed that the call was remarkably high in the evening after rainfall.

The notes lasted 0.5 s and were composed a single pulse. The notes interval ranges from 0.9–10 s. The frequency spectra have a dominant band at 1981.055 Hz. The band width ranges from 359.5705 to 1570.255 Hz (Figure 1).

### *Mating and spawning*

In this study, vocalization of unsuccessful male was noticed from evening to the next morning. During the mating period, males first enter the breeding ground and started advertisement calls. In respond to males' vocalization, females entered the breeding pool from the surrounding forest. Female approached male slowly, there is no visual cue. Male then suddenly grasped the female resulting in axillary amplexus. Amplexus was observed to take place during both day and night. In the present study, combating behaviour of males was observed, where one male frog tried to dislodge another amplexing pair. Amplexing was axillary and within a day, on the 29<sup>th</sup> March 2015, a single pair was found in the study site I whereas two to three pairs were also encountered in the study site II. Amplexus was observed to last several minutes before the nest construction took place and the female deposited its eggs in a large white foam nest. During this study period, a pair of amplexing male and female in the study site II had spent about 41 minutes for the construc-

tion of a foam nest. The foam nests with a diameter of 10–13 cm were found adhering to the wall. Some of the foam nests constructed were attached to the grasses and stones on the side of the pond and also floating on the water surface. Both male and female frogs left the nests after construction and no parental care was observed. Fresh laid eggs were collected from the breeding sites and were monitored in the laboratory. The clutch size for *Polypedates teraiensis* ranges between 600–650 in the present study.

### *Morphometric measurements*

Morphometric measurements are given in Table 1.

## Discussion

It was observed that *Polypedates teraiensis* is a seasonal breeder, breeding only during the monsoon period. Depending on the rainfall the breeding season may be extended from March to September. Rainfall influences the reproductive phenology of many amphibian species, particularly in tropical forests with seasonal precipitation.<sup>17</sup> Therefore, dependency upon an aquatic environment for reproduction results in breeding migrations before and after spawning. It was suggested that only the onset of monsoon stimulates the animals to emerge from their subterranean retreats and strong choruses of breeding aggregations have been heard following prolonged non-violent rains which lasted several days. Prolonged droughts may completely prevent breeding and several continuous days with small rainfall may be as important in the breeding of amphibians as is a single day with heavy rainfall.<sup>18</sup> In the present study, the males of *P. teraiensis* were found to select a suitable spawning site closer to water body so that the tadpoles when hatched can fall into the water body for further development. Shepard<sup>19</sup> suggested that selection of the oviposition site may be the primary means by which parents can directly influence their offspring probability of survival in some species.

**Table 1 | Measurement of the amplexing frogs (in mm) were carried out using a dial calliper accurate to 0.01 mm. (N= Total number of frogs examined)**

Sl. No	Character	Males N=5		Females N=5	
		Range (mm)	Mean±SE	Range (mm)	Mean±SE
1	SVL	52.93 - 56.45	55.07 ±1.41	67.89 - 75.22	72.93 ±3.13
2	HW	15.15 - 16.79	15.86 ±0.61	19.73 - 22.78	21.74 ±1.18
3	HL	22.79 - 24.44	23.67 ±0.69	28.19 - 31.13	29.64 ±1.21
4	MN	20.14 - 22.45	21.26 ±0.86	26.39 - 30.33	27.97 ±1.52
5	MFE	15.31 - 16.51	16.12 ±0.47	20.32 - 23.28	21.75 ±1.37
6	MBE	10.23 - 11.81	11.09 ±0.65	13.06 - 17.81	15.30 ±1.71
7	IFE	10.94 - 12.46	11.09 ±0.22	13.34 - 15.89	15.17 ±1.09
8	IBE	15.9 - 17.15	16.45 ±0.51	19.65 - 22.90	21.45 ±1.20
9	IN	4.23 - 5.22	4.51 ±0.41	5.34 - 6.78	6.23 ±0.55
10	EN	5.34 - 6.77	5.96 ±0.62	6.31 - 8.16	7.38 ±0.67
11	EL	6.23 - 6.52	6.33 ±0.11	6.24 - 7.29	7.03 ±0.45
12	SN	3.01 - 3.84	3.29 ±0.34	3.48 - 4.34	3.95 ±0.32
13	SL	9.01 - 10	9.38 ±0.38	10.45 - 12.55	11.51 ±0.79
14	TYD	3.09 - 4.65	3.87 ±0.73	4.15 - 5.6	5.03 ±0.60
15	TYE	1.23 - 1.61	1.36 ±0.16	1.99 - 2.98	2.51 ±0.42
16	IUE	5.33 - 6.53	6.08 ±0.46	9.72 - 11.46	10.55 ±0.66
17	UEW	4.05 - 4.88	4.55 ±0.42	4.69 - 5.61	5.3 ±0.36
18	FLL	10.94 - 12.81	11.69 ±0.72	16.56 - 18.54	17.48 ±0.74
19	HAL	12.09 - 13.95	12.9 ±0.93	18.67 - 20.64	20 ±0.79
20	TFL	8.04 - 8.76	8.44 ±0.26	10.78 - 12.67	11.74 ±0.75
21	PA I	1.23 - 1.8	1.58 ±0.21	2.02 - 2.38	2.22 ±0.15
22	PA II	2.09 - 2.51	2.33 ±0.19	2.47 - 3.65	3.11 ±0.46
23	PA III	2.06 - 2.73	2.33 ±0.32	3.67 - 4.54	4.09 ±0.37
24	PA IV	2.03 - 2.82	2.44 ±0.32	3.89 - 4.76	4.4 ±0.41
25	WA I	0.12 - 0.92	0.36 ±0.33	0.77 - 1.23	1.04 ±0.19
26	WA II	0.01 - 0.96	0.43 ±0.36	0.93 - 1.96	1.51 ±0.42
27	WA III	0.06 - 0.89	0.5 ±0.3	1.04 - 2.34	1.58 ±0.49
28	WA IV	0.13 - 0.99	0.57 ±0.31	1.05 - 2.09	1.58 ±0.45
29	FL	26.49 - 30.67	28.14 ±1.61	35.55 - 42.61	38.75 ±2.86
30	TL	28.71 - 30.35	29.27 ±0.71	37.67 - 42.24	39.83 ±1.82
31	TFOL	22.51 - 23.47	22.97 ±0.37	30.67 - 32.77	31.95 ±0.92
32	FOL	22.91 - 23.72	23.22 ±0.35	30.54 - 32.92	32.07 ±1.04
33	FTL	16.08 - 17.24	16.58 ±0.47	21.56 - 23.78	23.05 ±0.92
34	PP I	1.08 - 1.78	1.37 ±0.26	2.01 - 2.79	2.38 ±0.34
35	PP II	1.38 - 1.92	1.59 ±0.22	2.76 - 3.56	3.15 ±0.29
36	PPIII	1.63 - 2.11	1.82 ±0.19	2.78 - 3.65	3.21 ±0.38
37	PPIV	1.8 - 2.05	1.94 ±0.09	2.99 - 3.78	3.53 ±0.31
38	PP V	1.63 - 2.17	1.91 ±0.22	2.87 - 3.35	3.15 ±0.18
39	WPI	0.11 - 0.8	0.52 ±0.29	0.92 - 1.43	1.21 ±0.19
40	WPPII	0.23 - 0.89	0.62 ±0.28	1.04 - 1.61	1.41 ±0.22
41	WPPIII	0.45 - 0.77	0.65 ±0.15	1.11 - 1.45	1.3 ±0.12
42	WPPIV	0.18 - 0.91	0.66 ±0.29	1.11 - 1.79	1.46 ±0.33
43	WPV	0.19 - 0.96	0.67 ±0.29	1.04 - 1.63	1.42 ±0.23
44	IMT	1.21 - 1.51	1.33 ±0.12	1.91 - 2.89	2.6 ±0.39
45	ITL	6.04 - 6.81	6.46 ±0.28	7.97 - 10.91	8.46 ±1.27
46	MTTF	12.98 - 19.43	13.19 ±0.17	17.39 - 19.42	18.45 ±0.79
47	TFTF	7.12 - 8.06	7.45 ±0.37	11.92 - 12.54	12.28 ±0.22
48	MTFF	14.04 - 15.55	14.68 ±0.77	20.78 - 22.4	21.84 ±0.79

49	FFTF	7.18-8.7	7.75 ±0.68	9.82-11.02	10.4 ±0.53
50	WTF	2.81-3.22	3.03 ±0.16	5.72-6.99	6.39 ±0.58
51	WFF	5.11-5.29	5.17 ±0.07	6.88-7.79	7.24 ±0.45
52	T1	7.08-7.65	7.32 ±0.23	11.67-12.67	12.06 ±0.37
53	T2	10.61-11.63	11.11 ±0.43	15.44-17.27	16.48 ±0.66
54	T3	15.74-16.72	16.13 ±0.04	22.71-24.54	23.82 ±0.72
55	T4	21.94-23.49	22.38 ±0.66	31.1-32.56	32.17 ±0.32
56	T5	17.71-19.73	18.68 ±0.75	25.82-27.49	26.79 ±0.65

Advertisement calls of *P. teraiensis* are short consisting of a single pulse, and have frequency spectra with a dominant band of 1981.005 Hz and the band width ranges from 359.5705 to 1570.255 Hz. The calls of other breeding species of *Polypedates* like *P. maculatus* are almost similar. Their calls are short consisting of a single pulse and the sound energy is distributed between 100-3900 Hz<sup>21</sup>. In anurans, spectral call properties, such as dominant or fundamental frequency, are usually negatively correlated with body size because of morphological constraints on the sound producing apparatus<sup>20</sup>. A number of factors have been invoked to explain geographic variation in frog calls including reinforcement changes in the acoustic environment or a divergence associated with morphological changes over the geographic range of the species.

From the present investigation it is found that females are larger (SVL = 74.58–75.22 mm) than males (SVL = 52.93–55.82 mm). Sexual dimorphism is represented by their sizes. The clutch size for *P. teraiensis* ranged between 600–650 in the present study. Chakravarty *et al.*<sup>21</sup> reported from Assam that the clutch size for *P. teraiensis* consists about 100 eggs and Tamuly and Dey<sup>22</sup> reported that the clutch size ranged between 100-150 eggs. Mohanty and Dutta<sup>23</sup> reported for *P. maculatus*, the number of eggs ranged from 275-719, whereas Girish and Saidapur<sup>24</sup> found the number of hatchling per nest ranged between 210-448 in *P. maculatus*. Mohanty-Hejmadi *et al.*<sup>25</sup> reported the number of eggs correlates with the female's nutritional state. Moreover, Ritke *et al.*<sup>26</sup> and, Morrison and Hero<sup>27</sup> reported that clutch size and breeding

phenology may vary over the geographic range of a wide-ranging species which may lead to variation in population dynamics.

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