

## ORIGINAL ARTICLE



# The breeding biology of *Fejervarya limnocharis* complex, *F. multistriata* (Hallowell, 1861) in Mizoram, northeast India

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The breeding behavior of *Fejervarya multistriata* was studied from the selected study sites in Mizoram for over three years between 2016 and 2019. In natural condition, breeding took place in group. The advertisement call of males consisted of a series of notes emitted at an interval of 0.1–0.4 s where the frequency spectra had a dominant band at 2670.1172 Hz and the band width ranges from 2497.8516–28422.3828 Hz. Morphometric measurements showed that females are larger than males with snout-vent length of  $45.55 \pm 1.54$  mm vs  $33.77 \pm 1.03$  mm, respectively. The clutch sizes ranges from 488 to 1035. We found that the calculated 't' value 0.03 is less than the 'p' value, i.e. 1, therefore there is no correlation between SVL of females and clutch sizes.

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## Introduction

The microglossid frogs of genus *Fejervarya* Bolkay, 1915 *sensu* Dubois & Ohler, 2000,<sup>1</sup> currently consists of 13 nominal species distributed in South and Southeast Asia.<sup>2</sup> Members of this genus are generally small to medium sized, semi-aquatic in habit and mostly live near the edge of ponds, marshes, rivers, forest streams and paddy fields.<sup>3</sup> However, the wide distribution and limited morphological differences among the species of this genus have created some confusion and difficulty, an often-various species of this genus belongs to *F. limnocharis* complex.<sup>4</sup> Being cryptic, mainly because of the conserved external morphological attributes,<sup>5–10</sup> taxonomic status for many of the species remains uncertain and hence, identification of this group currently aided by acoustic as well as genetic analysis.<sup>11–13</sup> One of the most frequently encountered 'species' names in this genus in India, is *F. limnocharis* (Gravenhorst, 1829).<sup>14</sup> This species was first described as *Rana limnocharis* based on

material originating from the Sundaic Island of Java,<sup>15</sup> it is reported to be highly provisional and definitely known only from at Indonesia, Malaysia, Laos, Myanmar, Thailand south of the Isthmus of Kra, Cambodia, and Vietnam north to (presumably artificially) to the Chinese border (but not recorded within that country). Reports from areas outside of this region almost assuredly apply to other species.<sup>1</sup>

Out of the total 13 nominate species, only 2 species, *F. cancrivora* and *F. orissaensis* are recorded from India.<sup>16</sup> Recently, Lalbiakzuala and Lalremsanga<sup>17</sup> reported the occurrence of a paddy frog, *F. multistriata* (Hallowell, 1861)<sup>18</sup> in Mizoram, India for the first time which represents the extension range of its distribution from subtropical and tropical China (from Yunnan and Guizhou through Guangdong to Hong Kong and Hainan) and Taiwan, and presumably also including populations in adjacent Vietnam, Laos, Thailand, and Myanmar.<sup>1</sup> In this paper, we present the breeding biology of *F.*

*multistriata* during their breeding season in Mizoram.

## Materials and Methods

### Breeding behaviour

To know the breeding season and breeding sites of *F. multistriata*, survey was conducted from June 2016 to August 2019. After surveying different water bodies in and around Tanhril village, Mizoram, India, two study sites were selected. In order to distinguish them from their sympatric species, *Minervarya asmati* in the field, all the snout-vent length (SVL) and other characters in ratios of the individuals were compared with the description of this species in Yang *et al.*<sup>19</sup> and Fei *et al.*<sup>20</sup>

**a) Study site I:** It is a natural field at Tanhril village where few grasses were grown in shallow puddles of stagnant water, with a GPS location of N 23°44.274': E 092°40.466' at an elevation of 964 m asl and N 23°44.291' and E 092°40.463' at an elevation of 949 m asl (Figure 1).

**b) Study site II:** It is an artificial pond located near Mizoram University (MZU) Gate no. 2 which is in the area of Tanhril village, with a GPS location of 23°44.250' N and 092°40.396' E at an elevation of 929 m asl (Figure 2).

The breeding behaviours were monitored in the field and to study their clutch sizes, amplexing pairs were collected from different study sites and kept in a separate glass container of 250 ml or 500 ml volume (depending on their sizes) covered with nets and brought to the laboratory. This method is very helpful for monitoring the durations of amplexus and time spent for laying of eggs by female adult. After the eggs were laid in the laboratory, they were maintained in a plastic tray containing pond water to allow further development and metamorphosis. The duration of the breeding pairs remained in amplexus were noted down and the pH and temperatures of atmospheric as well as water were recorded.

### Acoustic analysis

The advertisement calls were recorded and analysed with the help of digital voice recorder Sony ICD-PX440 Professional compact voice recorder without external directional microphone which were usually held 30–60 cm away from the animal being recorded. The sampling used to convert the signals to digital format was 8 kHz with 16-bit precision. The oscillogram was prepared and analyzed with the help of a software tool "Sound Ruler 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.<sup>21</sup> The data were analyzed with the help of statistical software tools SPSS (7.5.1 version) and Origin Pro 8 SRO (8.0724 version).

### Morphometric measurement

The morphometric measurement of the amplexing pairs (males and females) were measured using a Mitutoya dial caliper accurate to 0.02 mm. While majority of the frogs were released back to the fields, some were killed by anesthetization with chloroform and then fixed in 5% formaldehyde and catalogued in the departmental Museum of Zoology, Mizoram University (MZMU). A small incision was made on the lateral side of the abdomen for proper preservation. Specimens were sexed either according to their external characters (in case of adult breeding males) or through a slight lateral incision in order to examine the gonads. Morphometric measurements largely follow the combination of Chanda,<sup>22</sup> Bain *et al.*,<sup>23</sup> and Ohler.<sup>24</sup>

### Abbreviations

**MZMU:** Departmental Museum of Zoology, Mizoram University, **SVL:** Snout-vent length, **HW:** Head width, **HL:** Head length, **MN:** Distance from the back of mandible to the nostril, **MFE:** Distant from the back of the mandible to the front of the eye, **MBE:** Distant from the back of the mandible to the back of the eye, **IFE:** Distant between the front of the eye, **IBE:** Distant between the back of the eye, **IN:** Inter nasal space, **EN:** Eye to nostril (distance from the front of the eye to the nostril), **ED:** Eye diameter, **SL:** Snout length (distance from the front of the eye to the tip of the snout), **SN:** Snout to nostril (distance from the nostril to the tip of snout), **TYD:** Greatest tympanum diameter, **TYE:** Distance from tympanum to the back of eye, **IUE:** Minimum distant between upper eyelids, **UEW:** Maximum width of inter upper eyelids, **FLL:** Fore limb length (from proximal end of arm with to tip of longest finger), **HAL:** Hand length (from the base of outer palmar tubercle to tip of finger), **TFL:** Third finger length, **PA:** Width of pads of fingers, **WA:** Width of fingers, **FL:** Femur length, **TL:** Tibia length, **TFOL:** Length of tarsus and foot, **FOL:** Foot length, **FTL:** Fourth toe length, **PP:** Width of pads of toes, **WP:** Width of toes, **IMT:** Length of inner metatarsal tubercle, **ITL:** Inner toe length, **MTTF:** Distance from the distal edge of the metatarsal tubercle to the maximum incurvation of the web between third and fourth toe, **TFTF:** Distance from the maximum incurvation of the web between third and fourth toe to the tip of fourth toe, **MTFF:** Distance from the distal edge of the metatarsal tubercle to the





**Figure 1** | Study site 1 which is a natural field located at Tanhril village.



**Figure 2** | Study site 2, an artificial pond located near MZU Gate no. 2.

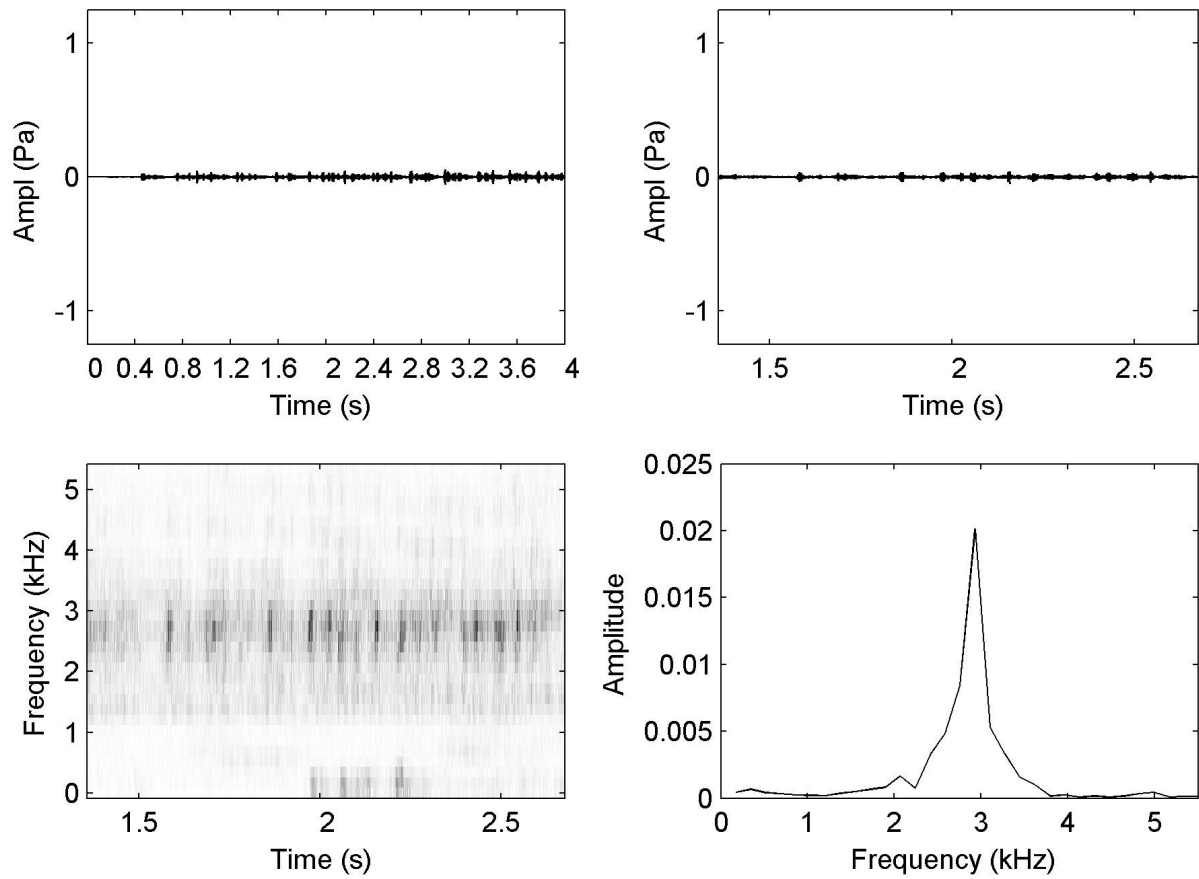
maximum incurvation of the web between fourth and fifth toe, **FFTF**: Distance from the maximum incurvation of the web between fourth and fifth toe to the tip of fourth toe, **WTF**: Webbing between third and fourth toe (from the base of the first subarticular tubercle), **WFF**: Webbing between fourth and fifth toe (from the base of the first subarticular tubercle), **T1** : From base of foot to tip of longest toe, **T2**: From base of foot to tip of

second toe, **T3**: From base of foot to tip of third toe , **T4**: From base of foot to tip of fourth toe , **T5**: From base of foot to tip of fifth toe.

## Results

### *Breeding behaviour*

During the present study, in respond to the first



**Figure 3** | Oscillogram, sonogram and frequency spectrum of an advertisement call of *Fejervarya multistriata*.



**Figure 4** | Amphiplexing pairs kept in separate container covered with net.



monsoon shower of March, males start callings from around 6:30 P.M. onwards in the study sites. Males usually call from water and from the ground in the vicinity of water. In respond to the male advertisement calls, female came out to the breeding ground from their hidden spots and individuals were encountered at around 9:00-10:30 P.M. The atmospheric temperature recorded ranged between 24 to 28°C, water temperature between 24 to 27°C, and pH between 5.54-7.22 during the investigation period in both the study sites till the month of August 2019. A few signs of breeding activities were also documented during the months of September and October.

### *Courtship and advertisement calls*

The advertisement call recorded at an ambient temperature of 25 to 27 °C consists of a long series of partially pulsed notes. Advertisement calls were emitted in series with variable call intervals. The call consisted of a series of notes (Figure 3) emitted at an interval of 0.1-0.4 s. The notes lasted 0.2-0.4 s and were composed of a series of pulses. The amplitude of the note increased smoothly from its two fifth, increased quickly before three and decrease before four. The frequency spectra had a dominant band at 2670.1172 Hz and the band width ranges from 2497.8516–28422.3828 Hz.

### *Mating and spawning*

In this study, male continued to call until female recognized their location. Once the female reached the male, suddenly male climbed on the back of

female and clasped from the back. Amplecting is axillary and amplecting pairs were encountered at around 11:00 P.M. to 12:00 A.M. and there was no visual cue for recognition. Breeding takes place in group and sometimes 3 to 5 pairs were encountered in the same water bodies within about 3 meter square. Males which do not take part in amplecting continued to calls till dawn even up to 4:30 A.M. to 5:00 A.M. Calls might be heard even during day time. Amplecting pairs were collected from the field and then transferred to another glass container along with some water from the study sites for laying their eggs and were covered with a net (Figures 4 & 5). It was found that females start to lay their eggs at around 2:00 A.M. to 3:00 A.M. That means amplexus lasted for 2 to 3 hours roughly in the laboratory condition. In the field, eggs were laid in various types of still waters, including paddy fields, ponds and rain pools. Egg masses were sunk to the bottom or attached to weeds in a small clump. The number of eggs (clutch size) range from 488–1035 (Figure 6). Morphometric measurements of amplecting males and females were measured.

### *Morphometric measurement*

Ten individuals of each male and female were measured (Table 1). It is found that females are larger ( $SVL = 45.55 \pm 1.54$  mm) than males ( $SVL = 33.77 \pm 1.03$  mm). Sexual dimorphism is represented by their sizes.

SVL of amplecting female ranges from 43.6–48.1 mm and their clutch sizes range from 488–1035 (Table 2). The correlation between SVL of females and clutch sizes is calculated by the following:



**Figure 5** | Axillary amplecting pair.



**Figure 6** | Freshly spawned eggs in the lab.

**Table 1 |** Morphometric measurements of males and females *Fejervarya multistriata* (N= Total number of frogs examined).

Sl. No.	Male	Range (N=10) (in mm)	Mean $\pm$ SE	Female	Range (N=10) (in mm)	Mean $\pm$ SE
1.	SVL	32.1 - 44.5	33.44 $\pm$ 0.33	SVL	45.6 - 48.1	45.55 $\pm$ 0.49
2.	HW	10.3 - 12.8	11.63 $\pm$ 0.28	HW	13 - 17.4	15.44 $\pm$ 0.49
3.	HL	13 - 14.2	13.43 $\pm$ 0.11	HL	15.2 - 20.2	17.55 $\pm$ 0.42
4.	MN	10.2 - 12.9	11.43 $\pm$ 0.25	MN	14.7 - 17.7	15.56 $\pm$ 0.29
5.	MFE	7 - 10.9	9.14 $\pm$ 0.37	MFE	10.5 - 15	12.22 $\pm$ 0.51
6.	MBE	4.8 - 6.9	6.16 $\pm$ 0.23	MBE	6 - 12.6	8.37 $\pm$ 0.54
7.	IFE	5.1 - 6.8	5.74 $\pm$ 0.17	IFE	5.1 - 8.6	7.18 $\pm$ 0.32
8.	IBE	8.2 - 8.9	8.68 $\pm$ 0.07	IBE	7.1 - 11.5	10.21 $\pm$ 0.37
9.	IN	2.3 - 3.6	2.91 $\pm$ 0.16	IN	3 - 4.7	3.93 $\pm$ 0.19
10.	EN	2.9 - 3.7	3.11 $\pm$ 0.17	EN	3 - 4.7	3.96 $\pm$ 0.16
11.	EL	2.2 - 4.3	3.32 $\pm$ 0.25	EL	3.3 - 4.8	4.24 $\pm$ 0.16
12.	SN	0.3 - 2.5	1.96 $\pm$ 0.21	SN	1.3 - 4.2	2.86 $\pm$ 0.29
13.	SL	4.1 - 6.7	5.3 $\pm$ 0.22	SL	2.2 - 8.8	6.36 $\pm$ 0.55
14.	TYD	1.6 - 3.3	2.48 $\pm$ 0.16	TYD	3 - 4.2	3.77 $\pm$ 0.37
15.	TYE	0.6 - 1.4	1.41 $\pm$ 0.11	TYE	1.3 - 3.7	1.93 $\pm$ 0.21
16.	IUE	2.1 - 3.8	2.82 $\pm$ 0.19	IUE	1.5 - 3.8	3.04 $\pm$ 0.22
17.	UEW	2 - 3.7	2.46 $\pm$ 0.16	UEW	2.2 - 3.4	2.99 $\pm$ 0.14
18.	FLL	12.6 - 14.4	13.93 $\pm$ 0.18	FLL	12.1 - 18.7	17.48 $\pm$ 0.62
19.	HAL	6.4 - 10.8	8.01 $\pm$ 0.41	HAL	8.1 - 11.1	10.19 $\pm$ 0.26
20.	TFL	3.1 - 4.6	3.88 $\pm$ 0.18	TFL	4.2 - 10.3	5.56 $\pm$ 0.55
21.	PAI	0.3 - 0.6	0.45 $\pm$ 0.03	PAI	0.4 - 0.7	0.49 $\pm$ 0.03
	PAII	0.3 - 0.5	0.39 $\pm$ 0.03	PAII	0.4 - 0.7	0.49 $\pm$ 0.03
	PAIII	0.2 - 0.6	0.35 $\pm$ 0.04	PAIII	0.3 - 0.7	0.44 $\pm$ 0.04
	PAIV	0.2 - 0.5	0.35 $\pm$ 0.03	PAIV	0.3 - 0.6	0.35 $\pm$ 0.03
22.	WAI	0.3 - 0.6	0.36 $\pm$ 0.04	WAI	0.3-0.5	0.36 $\pm$ 0.02
	WAI	0.2 - 0.6	0.35 $\pm$ 0.05	WAI	0.3 - 0.5	0.36 $\pm$ 0.02
	WAI	0.1 - 0.6	0.3 $\pm$ 0.05	WAI	0.2 - 0.5	0.33 $\pm$ 0.03
	WAI	0.1 - 0.7	0.31 $\pm$ 0.06	WAI	0.2 - 0.5	0.33 $\pm$ 0.03
23.	FL	10.2 - 17.8	16.07 $\pm$ 0.75	FL	20 - 24.1	22.01 $\pm$ 0.55
24.	TL	12.7 - 20.1	17.8 $\pm$ 0.66	TL	20.4 - 26	23.74 $\pm$ 0.56
25.	TFOL	23.1 - 27.9	26.19 $\pm$ 0.51	TFOL	24 - 37.1	32.89 $\pm$ 1.29
26.	FOL	16.5 - 20.7	18.28 $\pm$ 0.43	FOL	17.1 - 36.1	24.67 $\pm$ 1.49
27.	FTL	8.4 - 11.6	10.23 $\pm$ 0.33	FTL	9.1 - 15.5	13 $\pm$ 0.68
28.	PPI	0.3 - 0.5	0.4 $\pm$ 0.02	PPI	0.3 - 0.7	0.49 $\pm$ 0.03
	PPI	0.2 - 0.6	0.39 $\pm$ 0.04	PPI	0.4 - 0.6	0.45 $\pm$ 0.03
	PPI	0.2 - 0.5	0.32 $\pm$ 0.02	PPI	0.3 - 0.4	0.37 $\pm$ 0.02
	PPI	0.2 - 0.4	0.31 $\pm$ 0.03	PPI	0.3 - 0.5	0.45 $\pm$ 0.02
	PPV	0.2 - 0.4	0.29 $\pm$ 0.02	PPV	0.3 - 0.5	0.37 $\pm$ 0.03
29.	WPI	0.2 - 0.4	0.31 $\pm$ 0.02	WPI	0.2 - 0.6	0.38 $\pm$ 0.04
	WPI	0.2 - 0.4	0.27 $\pm$ 0.02	WPI	0.3 - 0.5	0.34 $\pm$ 0.03
	WPI	0.1 - 0.4	0.24 $\pm$ 0.03	WPI	0.2 - 0.5	0.32 $\pm$ 0.03
	WPI	0.1 - 0.5	0.28 $\pm$ 0.05	WPI	0.3 - 0.4	0.36 $\pm$ 0.02
	WPV	0.1 - 0.5	0.25 $\pm$ 0.03	WPV	0.3 - 0.4	0.34 $\pm$ 0.03
30.	IMT	1.3 - 2.8	1.93 $\pm$ 0.20	IMT	2 - 2.7	2.36 $\pm$ 0.09
31.	ITL	2.5 - 5.1	3.91 $\pm$ 0.27	ITL	4.3 - 6.2	4.77 $\pm$ 0.18
32.	MTTF	8 - 11.7	10.34 $\pm$ 0.32	MTTF	10 - 11.4	12.56 $\pm$ 0.79

33.	TFTF	6.1 - 8.4	7.3 ± 0.26	TFTF	10 - 16.6	9.84 ± 0.49
34.	MTFF	9.4 - 11.7	10.65 ± 0.21	MTFF	8 - 15.7	13.76 ± 0.68
35.	FFTF	7.3 - 10.7	8.36 ± 0.35	FFTF	8 - 12.7	10.74 ± 0.38
36.	WTF	3 - 4.9	3.42 ± 0.18	WTF	3.3 - 5.3	4.51 ± 0.18
37.	WFF	2.8 - 3.9	3.28 ± 0.11	WFF	3.3 - 5.6	4.64 ± 0.22
38.	T1	3.3 - 5.3	4.58 ± 0.18	T1	4.6 - 7.8	6.67 ± 0.29
39.	T2	6.6 - 10.6	8.28 ± 0.41	T2	6.5 - 12.9	10.92 ± 0.64
40.	T3	11.1 - 14	12.24 ± 0.36	T3	13.3 - 18.4	16.71 ± 0.50
41.	T4	15.8 - 20	17.66 ± 0.55	T4	22 - 25.2	23.98 ± 0.33
42.	T5	11 - 15.2	12.57 ± 0.43	T5	12 - 18.2	16.68 ± 0.7

**Table 2 |** Snout-vent length of females and their clutch sizes.

Sl. No.	Museum No.	SVL of Females (in mm)	Clutch size
1.	MZMU 1113	45.4	1035
2.	MZMU 1106	48.1	574
3.	MZMU 1107	47.1	937
4.	MZMU 1051	45.6	488
5.	*	43.6	598
6.	MZMU 1052	46.2	575
7.	MZMU 1047	46.8	603

(\* not catalogued and released back)

**Table 3 |** Pearson correlations.

		SVL	Clutch size
SVL	Pearson Corr.	1	0.01785
	Sig.	--	0.9697
Clutch size	Pearson Corr.	0.01785	1
	Sig.	0.9697	--

$$t = r / \sqrt{1-r^2} \times \sqrt{n-2}$$

where, t = student 't'- statistics

r = correlation

n = no. of observations

$$\text{Therefore, } t = 0.01785 / \sqrt{1 - (0.01785)^2} \times \sqrt{7-2} = 0.03$$

Since, the calculated 't' value 0.03 is less than 'p' value, i.e. 1, therefore in the present study there is positively no correlation between SVL of females and clutch sizes.

## Discussion

From the present observation it was observed that *Fejervarya multistriata* is an early breeder, breeding is triggered by the first shower of monsoon rain from March following till August. The species

breeds after rainfall as there must be some standing water for the deposition of spawn. The recorded atmospheric temperature (24 to 28°C), water temperature (24 to 27°C), and pH (5.54-7.22) might be the optimal for their breeding success. Rainfall influences the reproductive phenology of many amphibian species, particularly in tropical forests with seasonal precipitation<sup>25</sup>.

The notes (call) duration (0.2–0.4 s) is much longer than the duration (0.16 ± 0.08), while the dominant frequency (2670.1172 Hz) falls within that of the dominant frequency (1456.28 ± 83.55 and 2893.48 ± 115.92 Hz) reported by Zhou *et al.* from China,<sup>26</sup> in which it was identified as *F. limnocharis*.<sup>1</sup> Grosjean and Dubois<sup>27</sup> reported that the call of *F. multistriata* is a short call (0.380 s) composed of groups of two multi-pulsed notes which are only a few notes emitted before a longer call separated by an interval of 1.53 s. The duration of notes averages 0.08 s and the intervals between them, 0.21 s. The note repetition rate is relatively high (5.26 notes per second). Each note consists of seven or eight large pulses (10–12 ms) which have a decreasing amplitude throughout the note. They also mentioned that there is no silence interval between two consecutive pulses and the dominant frequency is 1371 Hz. The short call and long call were also witnessed in the present study where the short call was not analyzed. Anurans emit a variety of sounds during the breeding season,<sup>26</sup> and several studies have shown that social as well as environmental factors influence some call characteristics, such as dominant frequency, number of pulses, duration, and repetition rate of the note.<sup>28-29</sup> Chen *et al.* found that call duration and syllable length were negatively correlated with environmental temperature.<sup>30</sup> In addition, individual variation in body mass and body size can also affect call traits.<sup>31-32</sup> Other studies have found considerable intraspecific variation in the advertisement call of frog species that inhabit broad geographic areas with a range of environmental and climatic conditions.<sup>33-36</sup> Geographic divergence in advertisement call structure can be associated with genetic subdivision.<sup>33,35</sup> Further research is required to clarify the relationship between calls and

behaviours.<sup>27</sup>

From the present investigation it is found that females (SVL = 45.6–48.1 mm, with mean  $45.55 \pm 0.49$ ; N = 10) are larger than males (SVL = 32.1–34.9 mm, with mean  $33.44 \pm 0.33$ ; N = 10) which shows that sexual dimorphism is represented by their size. Matsui *et al.*<sup>13</sup> and Djong *et al.*,<sup>37</sup> reported from Japan that males SVL = 40.4–44.4 mm (average  $42.3 \pm 2.0$ ; N = 4) and females 44.6 – 52.4 mm (average  $47.2 \pm 2.9$ ; N = 13) which is quite similar with the size of females, but variation in males from the present study. During the present study male usually calls from water and in the vicinity of water and continued to call till female recognized the location. Amplecting is axillary, there is no visual cue and breeding takes place in group. From the observation, it was found that amplexus lasted for 2 to 3 hours roughly, eggs are laid in several small masses and are medium sized, enclosed in double jelly capsule, and the number of eggs range from 488–1035. Kuramoto<sup>38</sup> reported the congener species, *F. kawamurai* from Japan that the mean ( $\pm$  SE) clutch size is  $1244.6 \pm 154.9$  (SE, n = 19) and the ovum diameter  $1.13 \pm 0.032$ . The animal hemisphere of egg is light brown in color while the clutch size is  $706 \pm 57.117$  (SE, n = 10) and ovum diameter  $1.53 \pm 0.03$  in the present investigation. In the present study, with the help of statistical analysis, student 't'-statistics and Pearson correlations, it is found that there is no positively correlation between the SVL of females and clutch sizes, where 'p' value is 1.

The paddy or rice frog is a dominant amphibian and common prey for many animals like, fishes, snakes and birds. This predator pressure is thus considered an evolutionary dynamic that promotes and maintains *F. multistriata* polymorphisms.<sup>19</sup> May be because of these polymorphisms, many workers were confused *F. multistriata* with *F. limnocharis* in identification (see Frost)<sup>1</sup>. Nowadays, several stressors, such as habitat defragmentation, environmental pollution and anthropogenic activities have caused amphibian populations to rapidly decline. Meanwhile, increasing urbanization has a significant impact on natural ecosystems and presents a major threat to anuran populations<sup>39</sup>. Therefore, it is necessary to study and monitor the natural history of these important animals for designing protective measures.

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## Conflict of interest

The authors declare that there are no known conflicts of interest.

## Declaration of authors' contribution

The authors contributed their time and effort equally.

## References

1. Frost, D. R. (2020). Amphibian Species of the World: An Online Reference. Version 6.0 (Accessed on 11 October 2019). Electronic Database accessible at <http://research.amnh.org/herpetology/amphibia/index.html>. American Museum of Natural History, New York, USA. doi.org/10.5531/db.vz.0001.
2. Dubois, A., Ohler, A. (2000). Systematics of *Fejervarya limnocharis* (Gravenhorst, 1829) (Amphibia, Anura, Ranidae) and related species. 1. Nomenclatural status and type-specimens of the nominal species *Rana limnocharis* Gravenhorst, 1829. *Alytes*, **18**, 15–50.
3. Raj, P., Dinesh, K. P., Das, A., Dutta, S. K., Kar, N. B., Mohapatra, P. P. (2018). Two new species of cricket frogs of the genus *Fejervarya* Bolkey, 1915 (Anura: Dicroglossidae) from the Peninsular India. *Records of the Zoological Survey of India*, **118** (1), 1–21. <https://doi.org/10.11646/zootaxa.3999.1.5>
4. Islam, M. M., Kurose, N., Khan, M. M. R., Nishizawa, T., Kuramoto, M., Alam, M. S., Hasan, M., Kurniawan, N., Nishioka, M., Sumida, M. (2008). Genetic divergence and reproductive isolation in the genus *Fejervarya* (Amphibia: Anura) from Bangladesh inferred from morphological observation, crossing experiments, and molecular Analyses. *Zoological Science*, **25**, 1084–1105. <https://doi.org/10.2108/zsj.25.1084>
5. Dubois, A. (1987 "1986"). *Miscellanea taxinomica batrachologica* (I). *Alytes*, **5**, 7–95.
6. Toda, M., Matsui, M., Nishida, M., Ota, H. (1998). Genetic divergence among southeast and East Asian populations of *Rana limnocharis* (Amphibia: Anura), with special reference to sympatric cryptic species in Java. *Zoological Science*, **15**, 607–613. [https://doi.org/10.2108/0289-0003\(1998\)15\[607:GDASAE\]2.0.CO;2](https://doi.org/10.2108/0289-0003(1998)15[607:GDASAE]2.0.CO;2).



7. Chanda, S. K. (2002). *Handbook of Indian Amphibians*. Published by Zoological Survey of India, pp. 1–335.
8. Daniels, R. J. R. (2005). *Amphibians of Peninsular India*. Published by Indian Academy of Science and Universities Press, Hyderabad, pp. 1–268.
9. Kuramoto, M., Joshy, S. H., Kurabayashi, A., Sumida, M. (2008 “2007”). The genus *Fejervarya* (Anura: Ranidae) in central Western Ghats, India, with descriptions of four new cryptic species. *Current Herpetology*, **26**, 81–105. [https://doi.org/10.3105/1881-1019\(2007\)26\[81:TGFARI\]2.0.CO;2](https://doi.org/10.3105/1881-1019(2007)26[81:TGFARI]2.0.CO;2).
10. Garg, S., Biju, S. D. (2017). Description of four new species of Burrowing Frogs in the *Fejervarya rufescens* complex (Dicroglossidae) with notes on morphological affinities of *Fejervarya* species in the Western Ghats. *Zootaxa*, **4277(4)**, 451–490. <https://doi.org/10.11646/zootaxa.4277.4.1>.
11. Dutta, S. K., Manamendra-arachchi, K. (1996). *The Amphibian Fauna of Sri Lanka*. Published by Wildlife Heritage Trust of Sri Lanka. Colombo, pp. 1–230.
12. Dutta, S. K., Singh, N. (1996). Status of *Limnonectes limnocharis* (Anura: Ranidae) species complex in Asia. *Zoos Print Journal*, **11(8)**, 15–21. <https://doi.org/10.11609/JoTT.ZPJ.1401.2639-4>.
13. Matsui, M., Toda, M., Ota, H. (2008 “2007”). A new species of frog allied to *Fejervarya limnocharis* from southern Ryukyus, Japan (Amphibia: Ranidae). *Current Herpetology*, **26**, 65–79. [https://doi.org/10.3105/1881-1019\(2007\)26\[65:ANSOFA\]2.0.CO;2](https://doi.org/10.3105/1881-1019(2007)26[65:ANSOFA]2.0.CO;2).
14. Gravenhorst, J. L. C. (1829). *Deliciae Musei Zoologici Vratislaviensis. Fasciculus primus. Chelonioset Batrachia*. Leipzig: Leopold Voss.
15. Ganesh, S. R., Dutta, S. K., Chandramouli, S. R. (2017). On the taxonomy and nomenclature of common Indian cricket frog *Rana Agricola* Jerdon, 1853 (Amphibia: Dicroglossidae). *Asian Journal of Conservation Bioogy*, **6**, 107–113.
16. Sanchez, E., Biju, S. D., Islam, M. M., Hasan, M. K., Ohler, A., Vences, M., Kurabayashi, A. (2018). Phylogeny and classification of fejervaryan frogs (Anura: Dicroglossidae). *Salamandra*, **54**, 109–116.
17. Lalbiakzuala, Lalremsanga, H. T. (2019). Geographic Distribution: India, Mizoram: *Fejervarya multistriata* (Amphibia: Anura: Dicroglossidae). *Herpetological Review* **52(2)**, 321.
18. Hallowell, E. (1861 “1860”). Report upon the Reptilia of the North Pacific Exploring Expedition, under command of Capt. John Rogers, U.S. *Proceedings of the Academy of Natural Sciences of Philadelphia*, **12**, 480–510.
19. Yang, D., Peng, L., Xu, J., Huang, S. (2017). Intraspecific polymorphism of rice frog, *Fejervarya multistriata* (Anura: Ranidae), in Lingnan, Huangshan, China. *Asian Herpetological Research* **8** (1), 22–26. <https://doi.org/10.16373/j.cnki.ahr.160036>.
20. Fei, L., Hu, S. Q., Ye, C. Y., Huang, Y. Z. (2009). *Fauna Sinica. Amphibia*, Vol. 2. Beijing: Science Press, 1310–1319 (In Chinese).
21. Lalremsanga, H. T., Hooroo, R. N. K. (2014). Bioacoustic analysis of the advertisement calls of *Kaloulapulchra* Gray, 1831 (Amphibia: Microhylidae) during the breeding season. *Science and Technology Journal*, **2(2)**, 13–20.
22. Chanda, S. K. (1994). *Memoirs of the Zoological Survey of India. Anuran (Amphibia) Fauna of North East India*. 18 (2). Zoological Survey of India, Kolkata.
23. Bain, R. H., Stuart, B. L., Orlov, N., L., (2006). Three new Indochinese species of cascade frogs (Amphibia: Ranidae) allied to *Rana archotaphus*. *Copeia*, **1**, 43–59. [https://doi.org/10.1643/0045-8511\(2006\)006\[0043:TNISOC\]2.0.CO;2](https://doi.org/10.1643/0045-8511(2006)006[0043:TNISOC]2.0.CO;2).
24. Ohler, A. (2007). Morphology and Morphometry of Frogs. *I<sup>st</sup> SERC School in Herpetology*. pp. 1–25.
25. Khongwir, S., Hooroo, R. N. K., Dutta, S. K. (2016). Breeding and nesting behaviour of *Rhacophorus maximus* (Anura: Rhacophoridae) in Meghalaya, North East India, *Current Science*, **110**, 1102–1105. <https://doi.org/10.18520/cs/v110/i6/1102-1105>.
26. Zhou, Y., Qiu, X., Fang, X., Yang, L., Zhao, Y., Fang, T., Zheng, W., Liu, J. (2014). Acoustic characteristics of eight common Chinese anurans during the breeding season. *Zoological Research*, **35**, 42–50. <https://doi.org/10.11813/j.issn.0254-5853.2014.1.042>.
27. Grosjean, S., Dubois, A. (2011). Description of the advertisement calls of nine species of *Fejervarya* Bolkey, 1915 and *Minervarya* Dubois, Ohler and Biju, 2001 from China, India and Nepal. *Alytes*, **27**, 117–141.
28. Heying, H. (2003). “Microhylidae” (on-line), Animal Diversity Web. Accessed February 13, 2008 at <http://animaldiversity.ummz.umich.edu/site/accounts/information/Microhylidae.html>.
29. Wells, K. D. (1988). The effect of social interactions on anuran vocal behavior. In: *The evolution of amphibian auditory system* (Fritsch, B., Ryan, M.J., Wilezynski, W., Hetherington, T.E. and Walkowiak, W. eds.) John Wiley and Sons, New York, pp. 433–454.

30. Chen, P., Zheng, R. Q., Huang, H., Yu, X. Y., Xu, Z.W. (2012). Mating call of *Paaspinosa* is related to temperature but not to body size. *Sichuan Journal of Zoology*, **31**(4), 513–517. (in Chinese)
31. Tárano, Z. (2001). Variation in male advertisement calls in the neotropical frog *Physalae musenesefae*. *Copeia*, **4**, 1064–1072.
32. Wang, J. C., Cui, J. G., Shi, H. T., Brauth, S. E., Tang, Y. Z. (2012). Effects of bodysize and environmental factors on the acoustic structure and temporal rhythm of calls in *Rhacophorus dennysi*. *Asian Herpetological Research* **3**(3), 205–212.
33. Ryan, M. J., Rand, A. S., Weigt, L. A. (1996). Allozyme and advertisement call variation in the tungara frog *Physalae muspustulosus*. *Evolution*. **50**, 2435–2453.
34. Nevo, E., Capranica, R. R. (1985). Evolutionary origin of ethological reproductive isolation in cricket frogs, *Acris*. *Evolutionary Biology*, **19**, 147–214.
35. Ryan, M. J., Wilczynski, W. (1991). Evolution of interspecific variation in the advertisement call of a cricket frog (*Acris crepitans*, Hylidae). *Biological Journal of the Linnean Society*, **44**, 249–271.
36. Hasegawa, Y., Ueda, H., Sumida, M. (1999). Clinal geographic variation in the advertisement call of the Wrinkled frog, *Rana rugosa*. *Herpetologica*. **55**, 31–324.
37. Djong, H. T., Matsui, M., Kuramoto, M., Nishioka, M., Sumida, M. (2011). A New Species of the *Fejervarya limnocharis* Complex from Japan (Anura, Dicroglossidae). *Zoological Science*, **28**, 922–929. <https://doi.org/10.2108/zsj.28.922>.
38. Kuramoto, M. (1978). Thermal tolerance of frog embryos as a function of developmental stage. *Herpetologica* **34**, 417–422.
39. Li, B., Zhang, W., Shu X, Pei E, Yuan X, Sun, Y., Wang, T., Wang, Z. (2016). The impacts of urbanization on the distribution and body condition of the rice-paddy frog (*Fejervarya multistriata*) and gold-striped pond frog (*Pelophylax plancyi*) in Shanghai, China. *Asian Herpetological Research*, **7**, 200–209.