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Observation on the breeding behaviour and analysis of the advertisement calls of *Rhacophorus maximus* Günther, 1858 (Anura: Rhacophoridae)

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Abstract

The breeding behaviour and the advertisement calls of the giant tree frog, *Rhacophorus maximus* was studied during the breeding season in Mizoram University Campus, Aizawl, Mizoram. At the first shower of the onset of monsoon, advertisement calls of male attract females to 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 analysis of advertisement calls of *Rhacophorus maximus.* Advertisement calls were emitted in series with variable call intervals. The morphometric measurement of the amplecting pairs were recorded and females are found to be larger than males.

Key words: Breeding behaviour, advertisement calls, *Rhacophorus maximus*, Mizoram.

Introduction

The diversity of reproductive modes in amphibians is higher than in any other vertebrate group.[1] One of the most interesting modes is that of rhacophorids, where majority of them deposit their eggs in foam nests, while others exhibit direct development.[2] The foam nests are thought to protect the eggs and embryos from predation and desiccation.[3,4] Construction of foam nests by Indian rhacophorids has been reported by many workers.[5–7] Anurans have a biphasic life cycle and they breed in a variety of water bodies such as temporary rainfed ponds, ephemeral pools, cemented tanks and permanent ponds. The breeding activity of rhacophorids like other anurans is influenced by the abiotic factors like temperature and rainfall.[8–10] Certain reproductive behaviour of many anuran species like the timing of their calling period are linked to climate.[11] Vocalization in amphibians is a common component of breeding behaviour as male anurans call to advertise their breeding status, defend territory and attract females.[12] The social

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behaviours of most anurans are associated with acoustic communication in the form of vocalization.[13] Advertisement calls are species specific. The intra-specific diversity in call characteristics allows females to discriminate among potential conspecific mates based on some of the same acoustic parameters used for species identification.[14]

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. Given the importance of acoustic signaling in the breeding ecology of most frogs, detailed acoustical and statistical descriptions of signals are an important first step toward understanding the reproductive and social behaviours of anurans. 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 non-invasive tool for the purposes of population census and monitoring. The integration of bioacoustic with other data sources can be important for effective conservation assessment, planning and management, especially for threatened and endangered species. Given the global decline in amphibians[15] integrating bioacoustic with anuran conservation is an important goal. Quantitative descriptions of animal vocalizations can inform an understanding of their evolutionary functions, the mechanisms for their utility in taxonomy, population monitoring, and conservation.[16]

Reviews dealing with various aspects or vocalization in rhacophoridae have been published by few workers, such as the calls of Bornean stream-breeding species of *Rhacophorus penanorum* , *R. kajau* , *R. belalongensis*, *R. angulirostris* and *R. gauni* were reported.[17-20] However, in northeast India, reports on the breeding behaviour and analysis of the advertisement call of any species under the genus, *Rhacophorus* is limited and there is no detailed study on the advertisement call of *Rhacophorus maximus. Rhacophorus maximus* commonly known as Nepal flying frog, Günther's tree frog or giant tree frog is listed as Least Concern by the IUCN Red List due to its extensive distribution and its tolerance of a wide range of habitat types.[21] It is a species of frog in the Rhacophoridae family found in southwestern China (Yunnan, Tibet), northeastern India, Nepal, western Thailand and northern Vietnam, and possibly in Bangladesh. Gupta *et al.*[22] reported that the breeding biology of *R. maximus* have not been studied in the field. Recently, Khongwir *et al.*[23] reported the breeding and nesting behaviour of this species in Meghalaya, northeast India, but the call analysis was not provided. Although it is known that like other rhacophorids, *R. maximus* construct foam nests to protect the eggs from desiccation during development there is scanty information on the breeding biology and the advertisement call of this tree frog. Therefore, the present investigation was carried out for a better understanding of its breeding and advertisement.

Materials and Methods

Study sites for Breeding Behaviour

During the study period, it was observed that *Rhacophorus maximus* was a seasonal breeder and its breeding activity coincides with the onset of monsoon i.e. late February to March in Mizoram. The study was conducted 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 1.8 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.

Morphometric Measurement

The morphometric measurement of the amplecting pairs (males and females) were measured using a dial caliper accurate to 0.01 mm, catalogued and preserved in 4% formalin in the Departmental Museum of Zoology, Mizoram University (MZMU).

Abbreviations used

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),**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, , **FLL:** Fore limb length (from proximal end of arm 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 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.

Acoustic analysis

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

RESULTS

Breeding season

In the present observation, the breeding activity of *Rhacophorus maximus* was stimulated by the first shower of monsoon rain in Mizoram. From February to March, the frogs came out to mate in the pools. The atmospheric temperature recorded during the investigation period ranges between 26 to 38ºC, water temperature between 24 to 28ºC, pH between 5.54- 8.22 in both the study sites.

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 (ca. 3:35 PM) and make advertisement calls onwards (Fig. 1). It was also observed that multiple males aggregate and produce advertisement call while hiding behind the grasses, some damp places near the breeding site, or while sub-merging on the water surface. Advertisement calls were audible to the human ear from a distance of about 30-35 m away. 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.

Advertisement calls were emitted in series with variable call intervals. The call consisted of a single note (Fig. 2) emitted at an interval of 0.8 -0.9 s. The notes lasted 0.4 s and were composed of a series of 2 pulses. The amplitude of the note increased quickly in its second third and decrease until the end. The frequency spectra had a dominant band at 1464.258 Hz and the band width ranges from 367.1037–692.2985 Hz.

Mating and Spawning

In this study, vocalization of unsuccessful male was noticed from evening to the next morning. During observation attracted by the calls, the female frog emerged and responded towards the breeding ground. On seeing the female, the male frog then emerged from its hiding place and encircled the female. It then suddenly grasped the female resulting in axillary amplexus (Fig. 3). Amplexus was observed to take place both during the day and night-time. In the present study, combating behavior of males was observed, where one male frog tried to dislodge another amplecting pair. Pairs in amplexus can be seen in water submerging together usually at the corner of the pool. Amplecting was axillary and a single pair was monitored in the study site I, whereas five pairs were also encountered in the study site II. Satellite males were also encountered near the amplecting pair (Fig. 4). They are found to breed in a group as sometimes three pairs of amplexus were encountered in the same breeding area of study site II. Amplexus was observed to last several hours before the nest construction took place and the female deposited its eggs in a large creamy white foam nest. The nests were observed to be constructed

Figure 1. Emerging male (MZMU 600) start calling from the breeding ground at study site II.

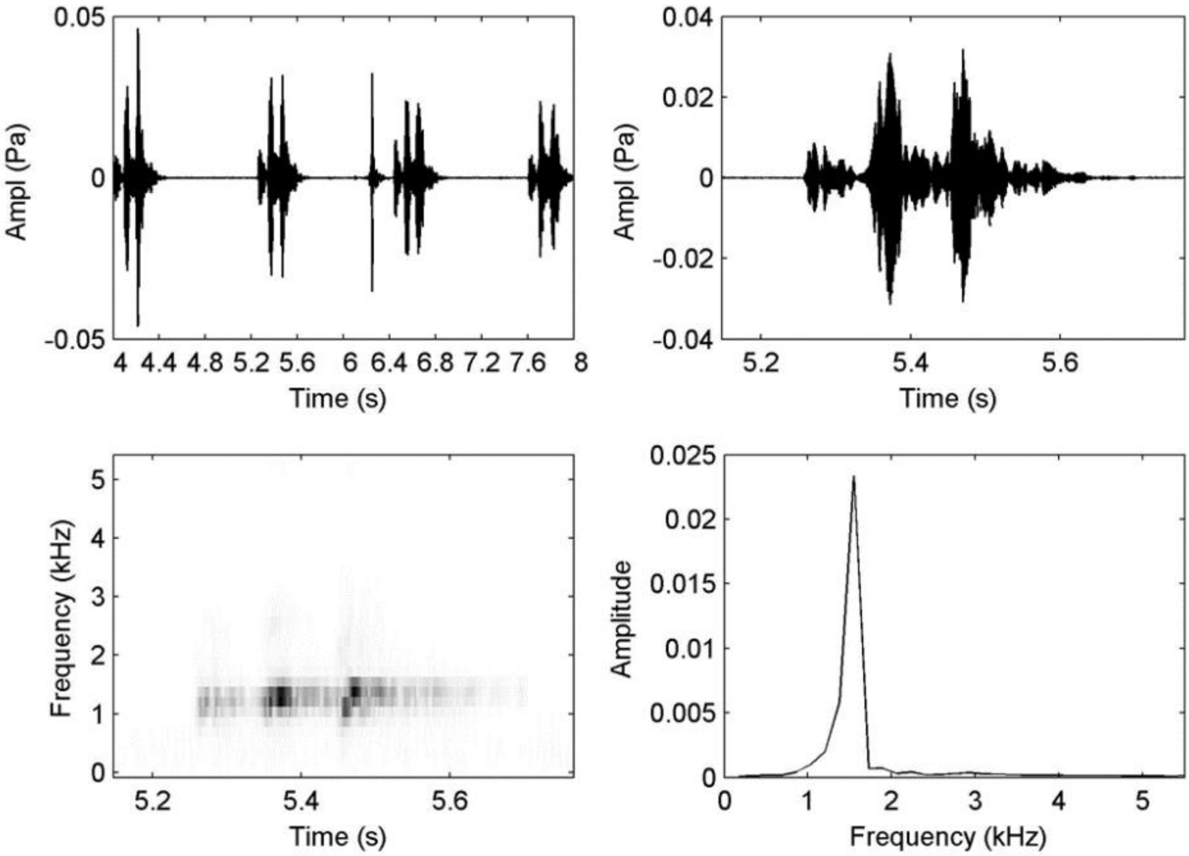

Figure 2. Oscillogram, sonogram and frequency spectrum of an advertisement call of male *Rhacophorus maximus* (MZMU 600).

Figure 3. Axillary amplecting in *Rhacophorus maximus* (MZMU 1104 and 1105).

Figure 4. Amplecting pair (MZMU 1106 and 1107) and satellite male.

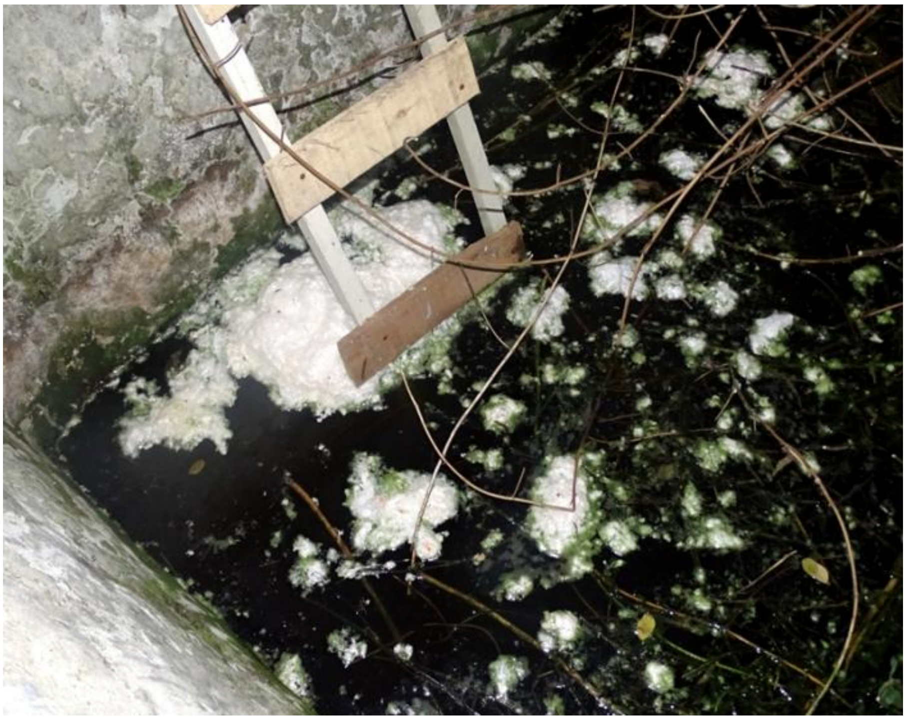

Figure 5. Foam nests of *Rhacophorus maximus* at study site I.

on diverse substrata. Some of the foam nests constructed were attached to the grasses and stones on the side of the pond and floating on the water surface (Fig. 5). 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 study sites are also utilized for breeding by other anurans like, *Duttaphrynus melanostictus* (Bufonidae), *Fejervarya limnocharis* (Dicroglossidae), *Kaloula pulchra* and *Microhyla ornate* (Microhylidae), and *Polypedates teraiensis* (Rhacophoridae) during their respective breeding season.

Morphometric measurements of the number of six (6) amplecting males and females are as shown in the Table 1.

Discussion

It was observed that *Rhacophorus maximus* is an early breeder, breeding activity is initiated with the first shower of the onset of monsoon, i.e. late February to March which is more or less similar with that of March to April at Cherrapunjee and Mawsynram in Meghalaya.[23] The observation of the present study agreed with Khongwir *et al.*,[23] where the species breeds after rainfall which leave standing water for the deposition of spawn. The breeding activity and movements of frogs to the temporary rain fed pools and ponds for spawning are initiated with an increase in temperature. Rainfall influences the reproductive phenology of many amphibian species, particularly in tropical forests with seasonal precipitation. 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.[23]

Construction of foam nest by *R. maximus* is suggested that to protect the eggs from desiccation due to changes in the external environmental factors during development. It is found that adult females of *R. maximus* are larger (SVL = 70.27-76.90 mm) than males (SVL = 47.88-70.14 mm). Sexual dimorphism is represented by their sizes. 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.[24] The advertisement calls of *Rhacophorus maximus* are strongly pulsed, short, consisting of 2 pulses, and have frequency spectra with a dominant band of 1464.258 Hz and the band width ranges from 367.1037 to 692.2985 Hz. The calls of another breeding species of *Rhacophurus* like *R. belalongensis* are very similar. They are strongly pulsed consisting of only 1-4 pulses, and have high frequencies with energy maxima between 3500 and 6000 Hz.[25] In constrast, the advertisement calls of similarly sized pond- breeding species like *R. rupifes* have comparatively much lower frequencies[20]. Several 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.[26-29] Further research on rhacophorids of NE India is needed so that more information about their evolution, ecology and reproductive strategies could be obtained.

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Table 1: Measurements of the mating frogs (in mm) (Dial caliper accurate to 0.01 mm)

Museum No.	MZMU 600	MZMU 601	MZMU 602	MZMU 1100	MZMU 1101	MZMU 1102	MZMU 1103	MZMU 1104	MZMU 1105	MZMU 1106	MZMU 1107	MZMU 1108
SEX	**M**	**M**	**M**	**F**	**F**	**M**	**F**	**M**	**F**	**F**	**M**	**F**
1. SVL	56.09	64.94	63.75	76.90	75.02	64.80	76.26	70.14	66.48	73.92	47.88	70.27
2. HW	19.24	22.46	24.84	26.17	27.69	22.80	26.97	23.55	20.67	24.8	15.24	23.23
3. HL	18.73	20.46	21.24	20.69	23.52	23.24	33.4	22.7	26.85	27.15	15.11	22.79
4. MN	16.44	17.27	16.92	15.53	19.66	21.42	27.79	19.01	21.23	21.48	11.27	18.81
5. MFE	10.87	11.63	11.24	11.54	13.37	16.42	22.99	13.77	14.36	14.84	8.64	11.93
6. MBE	4.84	6.57	4.11	6.77	6.88	7.64	16.14	5.62	8.99	6.52	2.08	6.14
7. IFE	10.59	12.22	13.21	14.93	14.02	14.55	16.01	13.67	13.34	14.88	10.8	18.74
8. IBE	11.72	17.76	20.07	20.59	20.53	21.70	24.8	20.53	19.53	20.46	15.86	20.4
9. IN	5.34	7.17	6.95	8.86	8.76	5.19	8.8	6.84	6.23	7.06	5.87	7.55
10. EN	3.05	5.4	4.76	5.36	6.78	5.62	4.6	6.97	5.04	6.01	5.67	4.59
11. EL	5.93	6.5	6.81	6.74	7.93	8.82	11.88	9.92	7.7	8.9	6.67	7.01
12. SN	3.88	4.49	4.77	5.42	5.21	7.44	8.43	5.91	5.16	6.04	4.9	6.73
13. SL	9.31	10.05	10.23	11.09	10.49	11.74	13.54	11.13	11.5	11.24	8.87	16.99
14. TYD	3.45	3.89	4.48	4.73	5.92	5.84	4.5	4.68	5.79	5.08	3.9	4.62
15. TYE	1.93	1.01	1.4	3.9	2.75	2.36	1.42	2.81	2.96	2.11	1.37	2.1
16. IUE	7.2	8.35	7.94	8.19	8.46	9.42	7.7	8.93	8.89	9.85	4.39	19.87
17. UEW	4.56	5.31	4.1	4.11	4.75	6.22	10.27	9.7	6.69	5.15	8.53	5.99
18. FLL	15.07	17.61	17.35	*	1O.04	16.34	19.26	18.2	16.04	17.66	12.24	15.89
19. HAL	14.91	16.93	18.36	*	18.43	21.34	19.2	17.92	15.73	18.14	10.07	17.23
20. TFL	5.9	12.75	11.79	*	12.78	13.64	15.31	12.36	13.01	10.47	8.19	13.44
21. PA I	1.51	1.98	2.33	*	2.29	3.24	2.61	1.55	1.5	2.09	1.14	1.13
PA II	2.72	3.04	3.93	*	4.03	5.24	4.53	4.79	4.9	3.56	3.77	4.37
PA III	3.38	4.32	4.68	*	4.56	5.94	5.27	4.41	4.41	5.99	3.92	5.08
PA IV	3.78	3.44	4.65	*	5.88	5.64	5.36	3.63	4.16	4.74	3.4	5.98
WA I	1.19	1.91	1.99	*	2.62	2.72	2.89	1.31	1.64	2.79	1.02	1.32
WA II	2.09	2.83	2.13	*	2.47	2.96	3.8	2.42	3.77	3.19	2.05	3.91
WA III	2.41	2.04	3.21	*	2.63	3.78	3.86	2.52	3.31	4.7	2.33	2.28
WA IV	2.43	3.17	3.34	*	3.93	3.74	3.99	2.29	3.84	4.56	2.9	3.79

22. FL	28.1	32.18	36.5	37.57	36.87	35.32	41.2	34.25	32.31	36.65	23.59	36.33
23. TL	30.98	33.17	33.98	36.09	34.74	35.06	41.21	33.56	33.31	37.5	23.78	35.89
24. TFOL	35.36	41.27	44.71	48.19	48.16	47.58	48.2	42.41	47.07	47.82	32.43	47.56
25. FOL	23.53	27.4	30.17	33.13	30.94	31.22	29.0	29.98	27.74	31.31	19.18	28.79
26. FTL	15.69	15.81	17.75	18.88	21.36	22.08	13.64	17.57	17.95	18.38	14.01	20.49
27. PP I	1.56	2.21	1.62	2.27	2.02	3.64	3.89	2.07	2.36	2.2	1.09	2.9
PP II	2.15	2.49	2.71	2.5	3.9	4.22	3.42	2.61	3.99	3.0	2.79	2.46
PP III	2.97	2.75	2.87	3.91	4.52	4.64	4.12	3.06	3.01	3.26	2.01	3.92
PP IV	2.85	3.53	3.36	4.56	5.27	4.72	5.88	3.21	3.48	3.56	3.72	3.63
PP V	2.7	3.64	2.95	3.77	5.88	4.98	4.11	3.85	3.95	2.57	3.73	3.02
28. WP I	0.87	1.71	1.36	1.58	2.2	2.66	2.91	1.3	2.77	1.47	1.2	2.63
WP II	1.5	1.82	2.57	2.71	3.52	2.84	2.64	2.79	2.05	2.2	1.56	2.41
WP III	1.84	1.05	2.73	2.01	3.6	3.32	3.37	2.07	3.83	2.53	2.68	3.88
WP IV	1.93	2.36	2.58	2.15	2.66	3.42	3.47	2.28	3.01	3.03	2.06	3.13
WP V	1.85	2.23	2.32	2.01	2.42	3.00	3.96	2.93	3.81	2.4	2.98	3.89
29. IMT	2.28	2.69	2.67	2.47	11.32	4.1	3.19	4.02	2.51	3.24	1.96	3.78
30. ITL	6.05	10.81	7.31	6.79	12.54	11.62	10.55	9.85	9.38	8.54	5.23	10.86
31. MTTF	16.59	20.44	20.49	25.56	26.84	17.28	26.21	20.79	22.25	23.08	15.03	22.57
32. TFTF	8.35	7.29	8.61	6.72	11.56	11.92	10.35	8.44	9.52	6.52	6.58	8.51
33. MTFF	17.77	21.51	22.13	27.13	7.09	26.26	27.19	22.95	22.11	28.8	18.8	24.06
34. FFTF	8.15	7.91	7.85	12.97	11.64	8.00	10.59	10.39	11.19	10.45	6.29	8.65
35. WTF	7.41	10.95	7.55	10.25	12.24	6.52	7.39	10.87	12.87	13.24	7.77	8.35
36. WFF	7.25	10.73	11.23	11.68	12.08	11.90	8.51	10.08	10.43	13.29	8.18	11.64
37. T1	6.8	8.83	9.14	11.12	12	10.93	9.22	9.77	10.95	9.97	5.23	10.82
38. T2	12.75	13.89	14.19	19.17	14.8	15.77	15.9	14.58	15.09	16.83	11.1	16.73
39. T3	18.39	20.79	20.95	25.27	22.56	22.62	24.2	23.06	23.42	23.74	18.08	18.71
40. T4	24.85	24.12	27.61	32.54	26.30	30.72	33.88	30.99	29.3	29.13	22.89	30.87
41. T5	22.5	22.67	24.24	29.99	28.34	27.13	29.63	23.68	26.8	27.89	20.76	27.68
42. TBW	7.22	7.45	9.23	9.4	9.42	5.55	9.68	6.75	8.41	8.43	5.94	19.8
43. TBL	30.63	33.4	34.31	32.8	36.52	34.08	41.03	33.32	35.24	37.14	23.12	34.05

* = Amputated limbs fed on by carnivorous tadpoles.

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