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Female release calls and male distress calls in the Southern Xinjiang Toad, *Bufotes pewzowi* (Bedriaga, 1898), from Uzbekistan

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Abstract. Amphibian vocalisations play a critical role in behavioural ecology, with release calls functioning as signals to deter undesired mating attempts and distress calls serving to mediate conflicts and signal imminent threats. Here, we provide the first description of female release calls and male distress calls of *Bufotes pewzowi* from Uzbekistan, Central Asia. Female release calls exhibited variability in both temporal and spectral properties, diverging from the release calls of males in other toad species. Male distress calls displayed similarities in temporal call property to those of *Rhinella granulosa*. Our study highlights the paucity of data on release and distress calls within the Family Bufonidae and the challenges in making comprehensive comparisons. Our findings contribute to an understanding of anuran communication and highlight the necessity for further research to elucidate the complexities of toad vocal behaviour.

Keywords. Amphibian communication, bioacoustics, Bufonidae, defensive behaviour, Central Asia

Introduction

Anuran vocalisations serve as crucial communicative signals and play a fundamental role in the behavioural ecology of anurans (Gerhardt and Huber, 2003; Toledo et al., 2015; Prasad et al., 2022). These calls, ranging from intricate mating choruses to territorial announcements, are pivotal for mate attraction, courtship rituals, territory establishment and signalling danger (Toledo et al., 2015; Köhler et al., 2017). For example, European Spadefoot Toads (genus *Pelobates*) emit distress calls as a defensive mechanism (Stănescu et al., 2018), while *Rhaebo guttatus* in Brazil emit release calls to prevent undesired amplexus from other males (Marinho et al., 2019). Release calls play a crucial role in preventing unwanted mating attempts, and their analysis contributes to our understanding of anuran social dynamics and reproductive behaviour (Duellman and Trueb, 1986; Tobias et al., 2014). Release calls in frogs represent a distinct subset of vocalisations that emerge in response to physical encounters, typically during aggressive interactions or amplexus attempts (Wells, 2007; Guerra et al., 2020). For example, four species of *Phyllomedusa* emit release calls when clasped by undesirable partners (Mângia et al., 2019).

Distress calls are an ancestral trait in anurans and show a phylogenetic signal, with greater similarity among related species compared to advertisement calls, reflecting evolution through convergent or neutral processes rather than divergent selection. Additionally, distress calls constitute a defensive vocal expression, emitted in response to imminent threats or predation (Forti et al., 2018). For example, tropical American hylid and leptodactylid frogs in Brazil emit distress calls to startle attacking predators and lure secondary predators (Hödl and Gollmann, 1986). These calls are characterised by their short call duration and their generally high or even ultrasonic frequency (Stănescu et al., 2018; Souza et al., 2024). As well as by their urgency and distinct acoustic features, such as high volume and likely serve as a means of alerting conspecifics and deterring potential predators (Hödl and Gollmann,

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1986; Forti et al., 2018). Understanding distress calls is paramount in unravelling the complex interplay between amphibians and their ecological surroundings (Forti et al., 2018; Souza et al., 2024).

Most vocal communication in anurans is conducted by males, with females remaining silent in most species. There are only a few species where females reciprocate with feeble calls during courtship, such as in *Xenopus laevis* (Tobias et al., 1998) and *Nyctibatrachus humayuni* (Willaert et al., 2016). In addition, there are a few known counterexamples, such as females of *Limnonectes blythii* and *Odorrana tormota*, which emit courtship calls to attract males (Emerson, 1992; Shen et al., 2008). Finally, females of a few species, such as *Colostethus inguinalis*, use territorial calls to defend their territories (Stewart and Rand, 1991; Pröhl, 2005). We here describe the release and distress calls of *Bufotes pewzowi* (Bedriaga, 1898).

Materials and Methods

Study species. *Bufotes pewzowi* is widely distributed across the arid plains of Central Asia, where it inhabits the foothills and mountains in Uzbekistan, Kazakhstan, Kyrgyzstan, and Tajikistan, as well as the mountainous and desert regions of western China and Mongolia (Fig. 1A; Litvinchuk et al., 2021). This species is a tetraploid, resulting from the hybridisation of *B. latastii* and *B. perrini* (Dufresne et al., 2019). In Uzbekistan, *B. pewzowi* inhabits the Gissaro-Alai Open Woodlands ecoregion of the mountains and foothills of the Fergana Valley and the Tien Shan Range, from high mountain slopes to urban areas. These toads breed from April to June, with egg strings laid in small waterbodies (AmphibiaChina, 2024). There is still very limited information available regarding the ecology and behaviour of this toad (Jablonski, 2017; Ikromov et al., 2023).

Field surveys. We conducted nocturnal visual encounter surveys in Oqtom Village, Fergana Valley, Uzbekistan (40.3245°N, 71.8183°E; 647 m elevation), where we found populations of *B. pewzowi* in agricultural and semi-natural habitats. During the survey, we captured several individuals, both male and female for morphological examination. Upon handling, three individuals produced vocalisations, prompting us to record their calls.

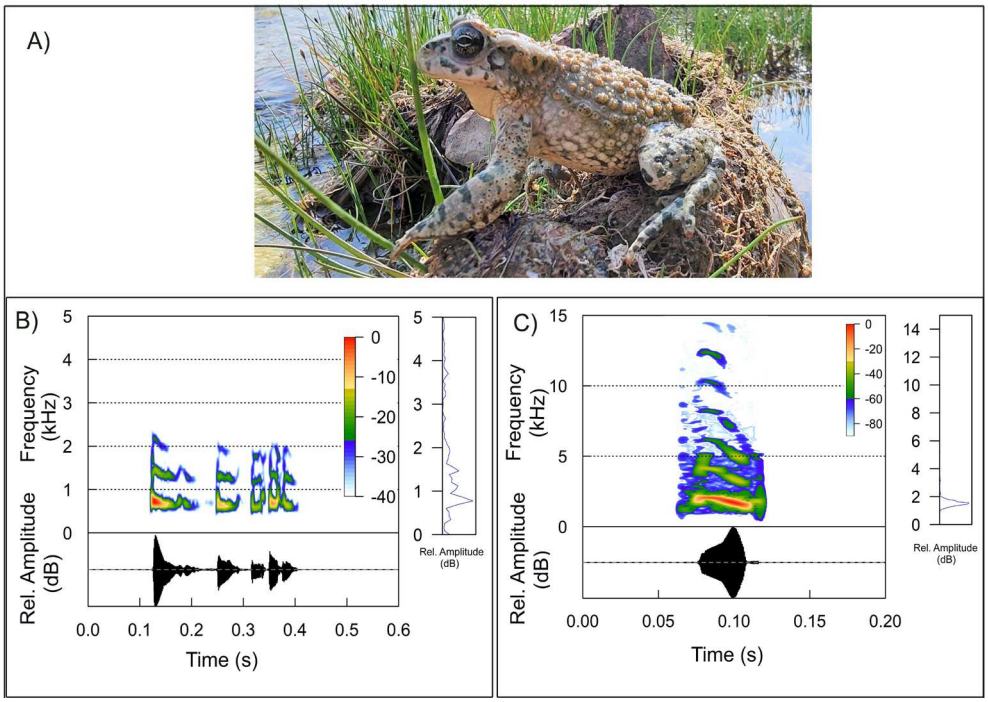

Figure 1. (A) A *Bufotes pewzowi* male from the Fergana Valley, Uzbekistan. (B) Female release call and (C) male distress call. Calls are depicted with coloured spectrograms (top), oscillograms (bottom), and power spectra (right). Photo by Amaël Borzée.

Data collection. To record the vocalisations, we temporarily collected one female and two males of *Bufotes pewzowi* between 17:00–18:00 h on 28 March 2023 near their natural habitat. When we gently stimulated individuals by holding them around the waist, the female emitted 17 release calls, whereas the two males emitted a total of 16 distress calls, with six and ten calls respectively emitted by each male. Males emitted distress calls while keeping their mouths open, in contrast with release calls. Shortly after the recordings were completed, we released all individuals at their original collection sites after measuring their snout-to-vent length (SVL) using digital callipers (CD-ASX, Kawasaki, Japan) to the nearest 0.1 mm. The recordings have been deposited to FonoZoo Sound Library (accession numbers 14686–14688, https://www.fonozoo.com/fnz_buscar_eng.php). We measured the air temperature at the call recording site using Extech En300 (USA) environment meter and recorded a temperature of 22.5 °C. We recorded calls utilising a linear PCM recorder (Tascam DR-40; TEAC America, Santa Fe Springs, California, USA) using the built-in microphone at a sampling rate of 44.1 kHz with 16-bit resolution. We manually adjusted the gain settings before starting the recording, and kept the settings constant. The toads were held 30–40 cm away from the microphones to avoid saturation while maintaining good quality.

Data analysis. We followed the recommendations of Koehler et al. (2017) when selecting eight call properties for our analysis using Raven Pro v1.5 software (Bioacoustics Research Program, 2017). Call properties included call duration (ms), rise time (ms), fall time (ms), low frequency (Hz), high frequency (Hz), sound intensity (dB), frequency modulation (Hz), and dominant harmonic (FFT size 1024 pts, Hanning window, resolution of 43.1 Hz). Duration, frequency, and intensity are provided as means ± standard deviation followed by their respective ranges. Definitions of these properties are provided in Table 1. The data extraction method followed Prasad et al. (2020, 2022). We generated oscillograms, spectrograms, and power spectrums using R software version 4.3.1 (R Core Team, 2021) using the packages *seewave* (Sueur et al., 2008) and *tuneR* (Ligges et al., 2014).

Results

Female release calls. We analysed 17 release calls from a single female toad (SVL 81.9 mm). These calls included multiple pulses, each with almost three harmonics in the 0.5–2.5 kHz range, of which the first carries the dominant frequency of the call (Fig. 1B). Relevant parameters are provided in Table 2.

Male distress calls. We analysed 16 distress calls emitted by two male individuals (SVL: 79 mm and 73 mm respectively). The distress calls were loud, tonal, and exhibited at least six harmonics per call, with the first harmonic carrying the dominant frequency (2.7–12.1 kHz; Fig. 1C, Table 2).

Call parameters for Male 1 (six calls) included call duration 41 ± 1 ms (26–57 ms), rise time 21 ± 1 ms (13–29 ms), fall time 21 ± 1 ms (13–28 ms), low frequency 1752 ± 259 Hz (1378–2039 Hz), and high frequency 3404 ± 837 Hz (2017–4190 Hz). The mean frequency modulation was 72 ± 423 Hz. (Table 2).

For Male 2 (ten calls), these values were 57 ± 26 ms (29–110 ms), rise time 29 ± 13 ms (14–55 ms), fall time 29 ± 13 ms (15–55 ms), low frequency 1672 ± 287 Hz (1300–2196 Hz), and high frequency 4040 ± 1894 Hz

Table 1. Temporal and spectral properties used to characterise calls of *Bufotes pewzowi* in Uzbekistan.

Call property	Description
Duration (ms)	Duration between the onset of the call and offset of the call.
Rise time (ms)	Duration between the onset of the call and point of peak amplitude in the call.
Fall time (ms)	Duration between point of peak amplitude in the call and offset of the call.
Low frequency (Hz)	Maximum frequency in the range of 1.3–2.2 kHz (low peak) determined over the duration of a call (FFT size = 1024 pts, Hanning window, 43.1 Hz resolution).
High frequency (Hz)	Maximum frequency in the range of 2.0–9.2 kHz (high peak) determined over the duration of a call FFT size = 1024 pts, Hanning window, 43.1 Hz resolution).
Intensity (dB)	The maximum power in a call.
Frequency modulation (Hz)	Difference between the low frequency and the high frequency.
Dominant harmonic	Energy concentration in the separately spaced frequencies of the longest wavelength wave.

Table 2. Key parameters of *Bufotes pewzowi* vocalisations in the Fergana Valley, Uzbekistan. Data include release calls from one female and distress calls from two males. Values are presented as mean ± standard deviation, with ranges provided in parentheses.

Call properties	Female Release call (*n* = 17)	Male 1 Distress call (*n* = 6)	Male 2 Distress call (*n* = 10)
Call duration (ms)	215 ± 66 (103–357)	41 ± 1.4 (26–57)	57 ± 26 (29–110)
Rise time (ms)	108 ± 33 (51–178)	20.5 ± 1 (13–29)	29 ± 13 (14–55)
Fall time (ms)	108 ± 33 (52–179)	21 ± 0.7 (13–28)	29 ± 13 (15–55)
Low frequency (Hz)	652 ± 161 (538–1255)	1751.6 ± 259 (1378–2039)	1671.7 ± 286.8 (1299.7–2196)
High frequency (Hz)	1305 ± 313 (1125–2487)	3404 ± 837 (2017–4190)	4040.3 ± 1893.9 (2554.6–9209.9)
Intensity (dB)	-5.6 ± 4.5 (-14–1)	-23 ± 13 (-39–0.7)	-28.8 ± 4.1 (-35– -22.3)
Frequency modulation	-58 ± 388 (-818–388)	71.8 ± 423.4 (-689–516.8)	25.8 ± 292.9 (-516.8–430.6)
Dominant harmonic	4 (3.5–4)	3.8 ± 0.6 (2.69–4.1)	5 ± 2.5 (3.8–12.1)

(2555–9210 Hz). The mean frequency modulation was 26 ± 293 Hz. (Table 2). Male 2 therefore had a longer mean call duration, rise time, and fall time, lower low frequency, and higher high frequency than Male 1.

Discussion

We provide the first description of female release calls and male distress calls for *Bufotes pewzowi*. The release calls produced by the female *B. pewzowi* showed notable distinctions in both temporal and spectral call properties compared to release calls of other toad species. For instance, male release calls of the northeast Asian *Strauchbufo raddei* exhibited shorter call duration, rise time, and fall time (Othman et al., 2022) than those of female *B. pewzowi*. A study from Argentina and Peru showed that male *Rhinella achalensis*, *R. arenarum*, *R. limensis*, and *R. spinulosa* also had shorter release call durations (di Tada et al., 2001). In Brazil (Vieira et al., 2014), male release calls of *R. mirandaribeiroi* and *R. dorbignyi* exhibited longer call durations, while those of *R. bernardoi*, *R. azarai*, *R. bergi*, and *R. fernandezae* displayed shorter release call durations than female *B. pewzowi*.

Our comparisons aim to provide a broader context for understanding the potential variations in toad release calls. While this approach may appear speculative, it is intended to highlight gaps in the existing literature and encourage researchers to explore the selective pressures and ecological factors shaping these vocalisations. Furthermore, female release calls generally exhibit a resemblance to those of males, as observed in *Bombina bombina*, where female and male release calls share similarities in call duration and overall structure (Gollmann et al., 2009). However, variations in female and male release calls are likely influenced by sexual dimorphism in the laryngeal apparatus, leading to the observed differences in frequency and amplitude in their vocalisations (Gollmann et al., 2009). However, there are very few studies to support this. Female *B. pewzowi* vocalisations have never been reported, and vocalisations in female anurans are generally rare (Zhang et al., 2017). Since male release calls have not yet been described for *B. pewzoni*, further fieldwork is necessary before any inferences can be drawn.

In male distress calls of *B. pewzowi*, the first harmonic carried the dominant frequency (Fig. 1C). This is consistent with the findings of Hödl and Gollmann (1986), who highlighted the generally high-frequency nature of distress calls. We observed a similar pattern, with call frequencies extending above 9 kHz (Table 2). Distress calls are known to be rich in harmonics (Forti et al., 2018; Stănescu et al., 2019), and this was also found in *B. pewzowi*, where we could identify six harmonics per call. These calls exhibited shorter durations compared to those of other toads. For example, *B. viridis* from Turkey and Austria, *Epidalea calamita* from Germany, *Rhinella granulosa* and *R. roqueana* from Brazil, had longer calls but displayed similar high-frequency components

(Weber, 1978; Hödl and Gollmann, 1986). The call duration of male *B. pewzowi* distress calls in our study was closely similar to that of *R. granulosa* (56 ± 38 ms; Hödl and Gollmann, 1986). However, the small sample size and the lack of published data, makes meaningful comparisons difficult. We suggest conducting further and more detailed studies on the acoustic behaviour of *B. pewzowi*.

Acknowledgments. This study adhered to the ethical guidelines of Institutional Animal Care and Use Committee and Ethics Committee of Nanjing Forestry University and is approved under obtained IACUC permit number 2023007. The calls were recorded without causing any harm to the animals involved, and all subjects received ethical treatment in accordance with the methodological guidelines given by the Animal Behaviour Society (Animal Behaviour Society, 2018). This study followed the laws of the Republic of Uzbekistan (https://lex.uz/docs/6821883), according to which the non-threatened species can be used for research without permission, and we complied with all relevant national, international, and institutional guidelines regarding animal care in Uzbekistan. We thank Obboskhon Azamov (Fergana State University) for his help during fieldwork. This project was funded by the Research Fund for International Scientists (RFIS) from the National Natural Science Foundation of China (NSFC; W2432021) awarded to AB.

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