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## Correspondence

# Description of female release calls of the European Common Frog, *Rana temporaria* (Anura: Ranidae)

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Anuran vocalization research has been conducted since the mid twentieth century, when calls were described and categorized according to their social context (Toledo et al. 2015). As a result, calls connected to mating and courtship are well studied, as they permit species recognition and are thus used for studies covering taxonomy, behavioural ecology, and monitoring programs (Wells 1977, Dorcas et al. 2009, Köhler et al. 2017).

The European Common Frog, *Rana temporaria* Linnaeus, 1758, is an explosive breeder with dense breeding aggregations in spring that are mainly male biased (Savage 1961). The advertisement call is not primarily addressed to the females, but to keep the chorus of males' clustered (Savage 1961, Van Gelder et al. 1978). Another function of the advertisement call could be the maintenance of the reproductive status in males, by keeping the androgen hormone levels high (Brzoska & Obert 1980).

The possibility of female mate choice in explosive breeding anurans is usually neglected (Wells 1977). Indeed, most publications state that female mate choice is precluded by scramble competition between males (Green et al. 2019) and those females are passive during the process of reproduction (Gollmann et al. 2014). Already in 1758, Rosenhof said that "then I have noticed that the female sometimes grunts too, but not so often and loudly"[translated from German]. Savage (1934) described Common Frog females producing grunting noises after deposition of their eggs, to signal their non-receptivity towards males. This publication lacked the description of the call, however. A release call of non-receptive females was described later by Brzoska et al. (1977) and is characterized by two frequency bands at 1100–1300 Hz and 1700–2000 Hz, respectively, and 18 pulses per call.

In this study, we describe two different release calls for *R. temporaria* females, compare them to the previously mentioned publications and discuss their possible behavioural context.

In spring 2019, we conducted behavioural experiments to investigate mate choice behaviour in *R. temporaria*. We performed fieldwork in the surroundings of the ecological field station of the University of Würzburg, in Fabrikschleichach (49.924 N, 10.555 E). We recorded experimental mate choice behaviour for one hour per experiment with a web camera with two internal microphones (Logitech C920) connected to a MacBook Pro. The webcam was attached to a tripod at 1.5 m height. The webcam settings permitted for a sampling rate of 44.1 kHz (247 kBit/s) and are recorded in a compressed mov-format. No filters or noise reduction have been used during recording; therefore, the recordings have a poor quality.

For the mate choice experiments, we put one male and two differently sized females in a plastic container (40 × 60 × 40 cm), filled with 10 l of rainwater (5 cm high). The containers were standing in the barn of the field station and air temperatures ranged from 5 to 15°C.

We converted those video sequences where males or females were calling into an audio wav-file (sampling rate: 44.1 kHz, 16-bit) and compared the calls to published spectrograms (Brzoska et al. 1977, Van Gelder et al. 1978). We down-sampled the wav-files in CoolEdit (sampling rate 11050 Hz, mono, 16-bit) and we used a bandpassfilter of 200–2000 Hz for the calls to remove background noise. If necessary, we removed background artefacts up to 300 Hz. We analysed the calls with Avisoft Bioacoustic software (Avisoft SASLab Pro Version 5.2.13, R. Specht, Glienicke, Germany). The configuration for the analyses was the following; FFT length: 1024, win-

Table 1. Properties of *Rana temporaria* female calls. Given are the female ID, which pair they belonged to, the females' snout–vent length in mm, the call type, average duration per single call in ms and the respective standard deviation, number of calls in a call series, average inter-call interval duration in ms, average minimum and maximum frequency from a call series and average dominant frequency of the call series (all in Hz).

| ID | pair | SVL (mm) | call type | duration (ms) | n calls | interval (ms) | minimum frequency (Hz) | maximum frequency (Hz) | dominant frequency (Hz) |
|---|---|---|---|---|---|---|---|---|---|
| 315 | 2 | 69 | squeak | 0.16 ± 0.01 | 5 | 1.21 ± 0.38 | 993 ± 69 | 1100 ± 74 | 1032 ± 92 |
| 349 | 5 | 65 | squeak | 0.15 ± 0.01 | 4 | 1.06 ± 0.38 | 824 ± 13 | 983 ± 36 | 953 ± 38 |
| 34 | 39 | 68 | squeak | 0.14 ± 0.02 | 10 | 0.73 ± 0.18 | 1105 ± 106 | 1285 ± 108 | 1222 ± 130 |
| 18 | 15 | 74 | squeak | 0.17 ± 0.03 | 8 | 2.84 ± 1.18 | 536 ± 196 | 1076 ± 37 | 985 ± 26 |
| 72 | 33 | 54 | squeak | 0.16 ± 0.04 | 6 | 2.23 ± 1.69 | 836 ± 42 | 1103 ± 206 | 951 ± 128 |
| 59 | 35 | 78 | squeak | 0.11 ± 0.04 | 5 | 1.55 ± 0.63 | 625 ± 125 | 977 ± 199 | 762 ± 36 |
| 8 | 18 | 53 | squeak | 0.15 ± 0.02 | 6 | 0.85 ± 0.51 | 1129 ± 41 | 1281 ± 26 | 1210 ± 52 |
| 35 | 31 | 71 | squeak | 0.15 ± 0.05 | 5 | 1.36 ± 0.88 | 859 ± 6 | 967 ± 49 | 884 ± 9 |
| 79 | 42 | 69 | squeak | 0.14 ± 0.02 | 5 | 1.19 ± 1.21 | 873 ± 272 | 1080 ± 106 | 954 ± 248 |
| 27 | 17 | 58 | squeak | 0.20 ± 0.02 | 11 | 0.95 ± 0.37 | 668 ± 116 | 1071 ± 33 | 914 ± 53 |
| 28 | 17 | 75 | squeak | 0.20 ± 0.02 | 4 | 0.97 ± 0.06 | 669 ± 24 | 1096 ± 73 | 884 ± 72 |
| 56 | 30 | 63 | squeak | 0.12 ± 0.02 | 17 | 0.52 ± 0.07 | 440 ± 187 | 1054 ± 82 | 826 ± 141 |
| 7 | 16 | 68 | squeak | 0.18 ± 0.03 | 12 | 1.27 ± 0.48 | 443 ± 53 | 1289 ± 68 | 802 ± 221 |
| 70 | 41 | 77 | squeak | 0.24 ± 0.04 | 13 | 0.73 ± 0.26 | 439 ± 48 | 1105 ± 229 | 699 ± 50 |
| 110 | 52 | 72 | squeak | 0.15 ± 0.03 | 9 | 2.32 ± 2.50 | 529 ± 91 | 946 ± 88 | 767 ± 101 |
| 92 | 46 | 65 | grunt | 0.17 ± 0.03 | 8 | 0.84 ± 0.23 | 435 ± 55 | 1124 ± 253 | 499 ± 27 |
| 107 | 49 | 74 | grunt | 0.20 ± 0.03 | 15 | 0.60 ± 0.11 | 237 ± 16 | 934 ± 90 | 295 ± 54 |
| 66 | 44 | 75 | grunt | 0.13 ± 0.03 | 4 | 2.28 ± 2.10 | 446 ± 6 | 958 ± 21 | 502 ± 69 |
| 58 | 34 | 75 | grunt | 0.18 ± 0.02 | 22 | 0.67 ± 0.14 | 364 ± 26 | 1180 ± 124 | 528 ± 134 |
| 12 | 23 | 63 | grunt | 0.16 ± 0.03 | 7 | 1.32 ± 0.88 | 330 ± 57 | 995 ± 161 | 588 ± 128 |
| 114 | 50 | 69 | grunt | 0.15 ± 0.02 | 12 | 0.88 ± 0.27 | 292 ± 20 | 889 ± 86 | 401 ± 153 |
| 26 | 13 | 86 | grunt | 0.21 ± 0.04 | 32 | 1.73 ± 1.29 | 263 ± 33 | 877 ± 28 | 403 ± 52 |

dow type: Bartlett, bandwidth: 56 Hz, resolution: 43 Hz, overlap: 93.75%, temporal resolution: 1.4512 ms. We measured the duration of the single calls, the number of calls in a call series, the inter-call interval, the minimum and maximum frequency and the dominant frequency per call. The spectrograms were drawn with R statistical software (R Core Team 2019, R version 3.6.1) and the packages seewave 2.1.4 (Sueur et al. 2008) and tuneR 1.3.3 (Ligges et al. 2018). We provide the parameters for the drawing of the spectrograms at the respective figures. The terminology and analysis of the call description follows the recommendations by Köhler et al. (2017). We analysed calls of two males (number of single calls, n = 12) and release calls from 22 females (number of single calls, n = 220). All sound files are deposited at the animal sound archive (https://www.tierstimmenarchiv.de/webinterface/contents/searchtext.php) of the Museum für Naturkunde, Berlin (archive numbers: Rana_temporaria_DIG0204_01–DIG0204_23).

The male calls fitted the known pattern and structure of the *R. temporaria* advertisement call, which is described having a frequency band between 300–900 Hz with a maximum frequency between 350–500 Hz, and two higher frequency bands at 1000–1400 Hz and 1400–1900 Hz (Brzoska et al. 1977). In our experiment 16 out of 41 males in amplexus called. This was in particular observed, when the female was moving and trying to free herself from amplexus. The male calls showed a dominant frequency of 521 ± 103 Hz (n males = 2, n single calls = 12, bandwidth: 296–1890 Hz, Fig. 1).

In our experiments, 26 females evoked calls and we were able to use 22 female calls for bioacoustics analyses (Table 1). Release calls are defined as audible calls emitted while tentatively amplected or touched by a male and are mostly coupled with little body side vibrations of the female (Toledo et al. 2015). We identified two distinct female release calls when females were amplected by a male, which were emitted directly after the male touched the female and whilst vibrations were observed on the flanks of the female. These release calls differed in their frequency distribution, but were similar in structure: a single unpulsed, non-frequency modulated simple call and a short duration of single calls in a call series. We defined all calls with an average dominant frequency below 600 Hz as a grunting sound and all calls with a dominant frequency above 600 Hz as a squeaking sound.

The first call type was a grunting sound with a dominant frequency of 459 ± 91 Hz (n females = 7, n single calls = 100), a minimum frequency of 338 ± 75 Hz and maximum frequency of 994 ± 108 Hz. The average duration of one single call was 0.17 ± 0.03 ms and average inter-call interval was 1.19 ± 0.62 ms. Females released 4–32 of these calls in a series. We provide the spectrogram and oscillogram of a grunting sound in Figure 2.

The second call type was a squeaking sound with a dominant frequency of 923 ± 146 Hz (n females = 15, n single calls = 120), a minimum frequency of 731 ± 227 Hz and maximum frequency of 1094 ± 108 Hz. The average duration of one single call was 0.16 ± 0.03 ms and average inter-call interval was 1.32 ± 0.66 ms. The females released 4–17 of these calls in a series. We provide the spectrogram and oscillogram of a squeaking sound in Figure 3.

The body size of an individual influences the frequency, that is larger animals produce lower frequencies (Wells 2007). We observed a significant negative correlation of snout–vent length and average dominant frequency (Fig. 4; Pearson correlation, n = 22, r = -0.49, p = 0.02). Individuals of similar body size were observed emitting both sounds, which indicates two different call types that are not simply depending on body size.

The calls of the males examined in our study corresponded in form and structure to the advertisement call described be Brzoska et al. (1977) and the B-call described by Van Gelder et al. (1978). In our experiments, these calls were emitted when the males grabbed a female and she was trying to free herself from amplexus. Savage (1934) stated that the male calling should keep the chorus clustered and is not addressed solely to the females. However, in our examples the call seems to be addressed to the struggling female only, and could be described as an amplectant call (Toledo et al. 2015). Therefore, we hypothesize that this call might also have a calming or comforting function towards the female.

The females emitted two different sound types that functionally seemed to be both release calls, but differed in their dominant frequencies. The squeaking sound with higher dominant frequencies seems to be the one described by Brzoska et al. (1977). The female grunting sound with lower dominant frequencies was more similar to the male release calls that show dominant frequencies around 200–300 Hz (Brzoska et al. 1977) and therefore, might have a different function than the squeaking release call. We as-

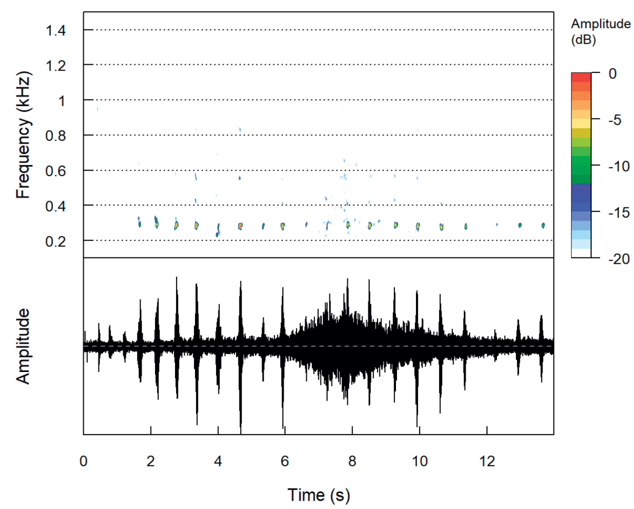


Figure 2. Spectrogram and oscillogram of *Rana temporaria* female ID107 (SVL: 74 mm; pair 49; TSA: Rana_temporaria_DIG0204_21) emitting grunting sound after being grabbed by a male. Dominant frequency; average ± SD: 295 ± 54 Hz; bandwidth: 237–934 Hz. Frequencies at second 6–10 are background noise. Spectrogram parameters: sampling frequency 11025 Hz, window length 1024, Hamming window.

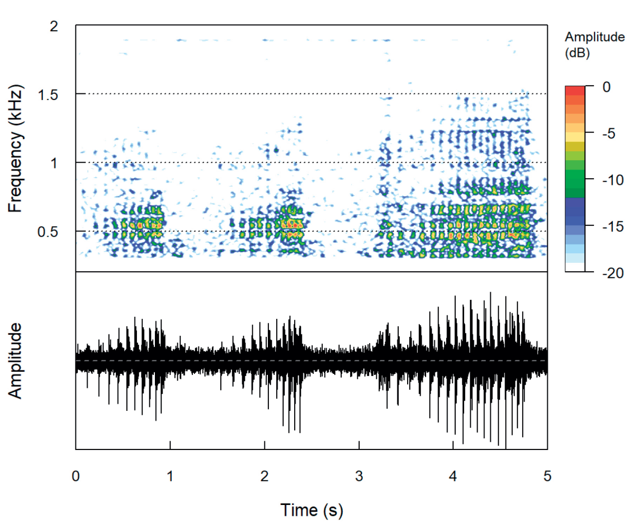


Figure 1. Spectrogram and oscillogram of a *Rana temporaria* male advertisement call (SVL: 62 mm; pair 44; Tierstimmenarchiv (TSA): Rana_temporaria_DIG0204_02). Dominant frequency; average ± SD: 492 ± 34 Hz; bandwidth: 293–1890 Hz. Spectrogram parameters: sampling frequency 11025 Hz, window length 1024, Hamming window, cut out second 9 to 14.

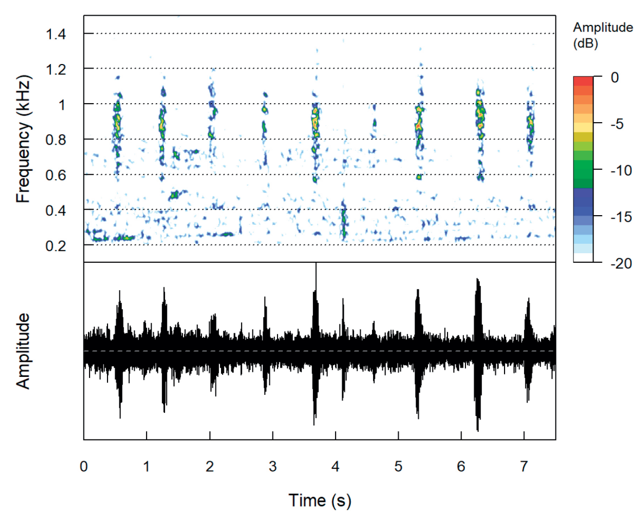


Figure 3. Spectrogram and oscillogram of *Rana temporaria* female ID27 (SVL: 58 mm; pair 17; TSA: Rana_temporaria_DIG0204_07) emitting squeaking sound after being grabbed by a male. Dominant frequency; average ± SD: 914 ± 53 Hz; bandwidth: 550–1148 Hz. Spectrogram parameters: sampling frequency 11025 Hz, window length 1024, Hamming window, cut seconds 3.5 to 11.

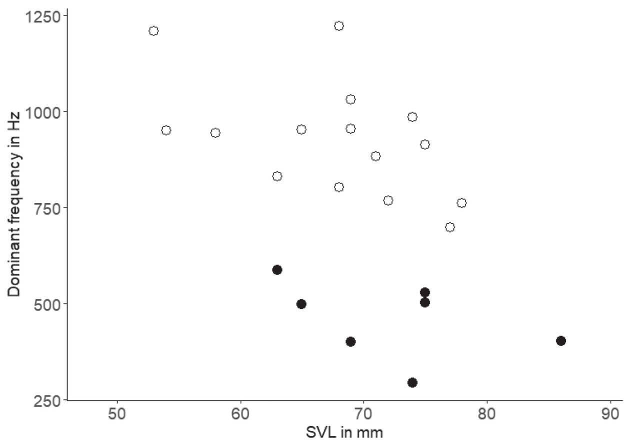


Figure 4. Snout–vent length in mm per *Rana temporaria* female and their respective average dominant frequency of the emitted female call in Hz. Dark dots represent the grunting sound, light dots the squeaking sound (compare text).

sume that the grunting release call might imitate the males' release call, and thus lead to a potentially faster release of females from amplexus, without spawning taking place. This hypothesis will be tested elsewhere. In support of our theory, the A-call described by Van Gelder et al. (1978) has similar dominant frequencies and lead more often to the release of other frogs than other call types. In addition, Savage (1961) described the male release call (he termed it "warning-croak") as a grunting sound and states that this sound is emitted by females as a signal for their un-readiness to mate (Savage 1934).

Herein, we have shown that female *Rana temporaria* may emit two different release calls when grabbed by a male, whereas Brzoska et al. (1977) had already described one with a higher dominant frequency, above 600 Hz. The second call, mentioned by Savage (1934), seems to imitate the male release call and may lead to a higher rate of successful escapes by females from amplexus.

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