

**The Vocal Repertoire of Marsh Frogs (*Pelophylax ridibundus*) on a Hungarian Danube
Island and Implications for Future Research and Conservation**

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ABSTRACT

In the past decade, extreme weather and anthropogenic habitat destruction led to considerable declines in amphibian populations around the world. One amphibian species affected by such changes are marsh frogs (*Pelophylax ridibundus*), water frogs found across Europe and Western Asia. Characterizing the vocal repertoire of marsh frogs may be helpful for their effective conservation; however, little systematic research has been done in this field. The current study aimed to identify and describe the vocal repertoire of marsh frogs on a Danube river island by combining quantitative analyses using stationary acoustic recordings with on-site qualitative field observations on the context of call emissions. Eight distinct call types were identified and evaluated using principal component analysis and discriminant function analysis based on maximum frequency. The call types were then compared to marsh frog vocalizations identified by previous studies in other geographic regions, and implications of the results were discussed. This study may prompt further research investigating potential semantic associations of anuran vocalizations, common patterns in amphibian communication, and the effects of anthropogenic noise on anuran vocal repertoires. Results of the study may also assist in the design of conservation-oriented technical devices for the identification of marsh frogs based on their acoustic characteristics.

Keywords: amphibian, marsh frog, animal communication, urban wildlife, acoustics

Abstract word count: 200 words.

INTRODUCTION

Animals use vocalizations in a variety of contexts including warning, mating, foraging, and social learning (Boinski & Mitchell, 1997; Freeberg, 2000; van Parijs et al., 2003; Seyfarth & Cheney, 2003). Animal vocalizations can serve a wide range of purposes, such as ensuring spatial cohesion of social groups, maintaining contact with conspecifics, signaling intra- and inter-sexual competition, moderating nonaggressive interactions between individuals, and alarming conspecifics of the degree of potential danger, and individuals can recognize calls emitted by conspecifics as a result of mechanisms such as natural and sexual selection in cryptic species and variations in the functional organization of hearing organs leading to the selective registration of auditory signals (Narins & Capranica, 1976; Jones, 1997; Suga, 1989; Owings & Morton, 1998; Fitch & Hauser, 2002; Leavesley & Magrath, 2005; Teixeira da Cunha & Byrne, 2009; Snowdon, 2018). Highly vocal animals comprise, but are not limited to, bats, primates, birds, ungulates, and frogs (Martof, 1961; Long et al., 1998; Engesser & Townsend, 2019; Fedorova & Shabanov, 2022).

Studying animal vocal communication may enhance our understanding of a variety of concepts and phenomena in evolutionary biology, ethology, and cognitive psychology. According to Hausberger (1993), examining the vocalizations of starlings (*Sturnus vulgaris*) in the field may help researchers raise questions about the learning, memory, and categorical perception abilities of the species (Hausberger, 1993). From an evolutionary perspective, the study of acoustic communication of field crickets (*Gryllus*), periodical cicadas (*Magicicada*), chorus and tree frogs (*Pseudacris* and *Hyla*), and tinkerbirds (*Pogoniulus*), and blind mole rats (*Spalax ehrenbergi*) has helped researchers study geographic variation and speciation (Nevo et al. 1987; Gerhardt, 2013). By studying the vocal interactions of the American bullfrog (*Rana*

catesbeiana), Boatright-Horowitz et al. (2000) were able to analyze intraspecific communication patterns of the species. Furthermore, by analyzing and measuring the total acoustic energy of American bullfrog vocalizations, Simmons et al. (2022) were able to estimate the chorusing activity of American bullfrogs surrounded by anthropogenic noise, potentially facilitating the estimation of population abundance and therefore the management of the species.

Marsh frogs (*Pelophylax ridibundus*) are medium-sized frogs widely distributed in Central and Southern Europe and Western Asia (Erismis, 2011). Their dorsal areas are gray- or green-colored and feature dark dorsal spots and often a light middorsal line, and their belly coloration usually includes gray, white, and yellow shades (**Figure 1**) (Kuzmin, 2022). Marsh frogs may co-occur with pool frogs (*Pelophylax lessonae*) and edible frogs (*Pelophylax esculentus*) in a given habitat, with the three species occasionally interbreeding (Mayer et al., 2013). During the breeding season, male individuals emit a wide range of loud vocalizations for territorial defense and mate attraction (Lukanov et al., 2013). During amplexus, female individuals lay clutches of eggs, with each clutch consisting of 670 to 13000 eggs. Metamorphosis of the offspring usually occurs from April to November, and fully developed individuals reach sexual maturity at the end of their second, third, or fourth year. Unlike the tadpoles that consume algae, detritus, plants, and animal material, fully developed marsh frogs feed primarily on terrestrial and aquatic insects. Marsh frogs can live up to 12 years in the wild (Kuzmin, 2022; Altun & Altunisik, 2024).



Figure 1: A vocalizing male marsh frog during breeding season in Hungary. While calling, male marsh frogs fill their gray vocal sacs with air – this helps in the amplification of their vocalizations (Bülbül & Zaman, 2022; M, 2018).

Marsh frogs are considered non-native or even invasive in countries such as the United Kingdom and France, and they are classified as Least Concern by the IUCN Red List (Pille et al., 2021; IUCN, 2023). However, in Hungary where they are native, and where all amphibians are threatened by climate change-induced droughts and habitat destruction, marsh frog populations have considerably declined over the past decade (Li, 2023; Kiss et al., 2019). Furthermore, a number of marsh frogs in the meadows of the Hungarian town of Kölked in Hungary were observed to develop deformed legs, abnormal numbers of limbs, and other defects potentially due to a high concentration of chemical pollutants in the water of the southern segment of the Danube river (Kiss et al., 2019). As their loud vocalizations can be detected over large distances, learning about the vocal repertoire of marsh frogs may aid the passive acoustic detection, and *in-situ* conservation and management of the species in and outside of Hungary (Dutilleux & Curé, 2020; Emmett et al., 2020).

Little research has been conducted on the vocal repertoire of marsh frogs. Fedorova and Shabanov (2022) and Lukanov et al. (2015) characterized specific call types, such as release and mating calls, and compared them to those of other frog species. However, these reports only focused on a specific call type in multiple species instead of multiple call types in one species. Focusing on investigating the vocal repertoire of marsh frogs, one study conducted by Lukanov et al. in 2011 recorded marsh frog calls from three locations in central and northern Bulgaria and identified a total of six call types: the “aggressive”, “distress”, “mating”, “release”, “territorial”, and “warning” calls¹ (see **Appendix B** for spectrograms of the six calls). To analyze the calls, they divided them into subcomponents called “pulses” and examined characteristics related to the timing, amplitude, and frequency of the calls, which they described as “pulse groups”. Although results of the study showed differences in the characteristics of the identified call types, statistical testing was only performed for the mean dominant frequency of the call types. Also, due to dividing the calls into ambiguously defined “pulses”, Lukanov et al. (2011) were not able to tell how many pulses of some of the call types were analyzed, making the sample size of these call types unknown.

Taking the limitations of Lukanov et al. (2011) into consideration, we aimed to study the entire vocal repertoire of marsh frogs with the purpose of documenting and describing call types both qualitatively and quantitatively, based on not only field descriptions of the calls but also extracted and analyzed acoustic data. To investigate the vocal repertoire of marsh frogs, we analyzed the acoustic structure of their vocalizations, and used statistical testing to determine the accuracy of our qualitative call classification. Based on the results of Lukanov et al. (2011), we predicted that marsh frogs on Margaret Island, the Danube island in Hungary where this study

¹ Unlike the original Lukanov et al. (2011) paper, I decided to place quotation marks around the behavioral functions (e.g., release) of the six identified call types, because the derivation of the behavioral functions associated with the call types is unclear in the paper.

was conducted, and potentially across Hungary use multiple call types to communicate with conspecifics in a variety of contexts. Based on existing literature, we also predicted that each marsh frog call type differs from other call types in both structural and temporal characteristics (Capranica, 1968; Vélez & Guajardo, 2020).

METHODS

Study Site

The field recordings and observations took place at a pond in the Japanese Garden of Margaret Island in Budapest, Hungary (47°32'04.0"N, 19°03'05.5"E, ~110 m elev.). The island was surrounded by the Danube River in central Budapest and is a popular site for tourism, recreation, and entertainment (**Figure 2a**). The pond was around 18 m long, 19 m wide, and 50 cm deep, and is surrounded by trees, a stone wall, pedestrian zones, and a wooden platform. The water of the pond was translucent with small amounts of plant remains and microorganisms on its surface. Fish, aquatic insects, tadpoles, metal coins, and pebbles were also present in the water. There were three or four small waterfalls pouring into the pond during the day, creating additional background noise. Since the pond is a tourist attraction on the island, there were a lot of visitors and vehicles around during the recording period. The pond was close to a bridge at the edge of the island connecting Buda with Pest, so vehicle noises from the bridge were also heard during the study. For a picture and satellite view of the pond, see **Figure 2a** and **2b**.



Figure 2: (a) A photograph of the Margaret Island pond taken on the wooden platform; (b) an annotated satellite image of the Margaret Island pond.

Acoustic Recording

Recording at this pond was carried out over the period 27/05/2022 – 30/05/2022 between 20:00 and 01:00 the following day on each of the four days. Twelve recordings with lengths between 30 and 60 minutes were made during this time, but only the first three recordings were used in the data analysis. For more detailed information about the time and the weather-related context of the recordings used in the data analysis, see **Appendix A**.

The recording device used in the study was an H4n Zoom audio recorder with two microphones enclosing a 120-degree angle set to a sampling rate of 44.1 kHz and a bit depth of 16 bits per sample. Throughout the study, the recorder was placed on the wooden platform 30 cm away from its edge (i.e., the location indicated by a red “X” mark in **Figure 2b**). The individual recordings were stored in an SD card in the H4n recorder, and they were saved onto a laptop as WAV audio files upon completion of data collection.

Species Identification

Prior to the study period, it was not known whether pool frogs and edible frogs were also present in the pond. Due to their similar physical appearances, distinguishing marsh frogs from edible frogs and pool frogs is often difficult (Blain et al., 2015). Therefore, identification of marsh frogs was conducted based on the distinct acoustic characteristics of their vocalizations² (Zamfirescu, 2004). During the study, no distinct edible frog or pool frog vocalizations were identified. However, in a few recordings, a small number of ambiguous vocalizations were found, the perceived acoustic characteristics of which resembled those of marsh frog and edible frog vocalizations (Wahl, 1969; Hoffmann & Reyer, 2013; Fedorova & Shabanov, 2023). To ensure the validity of the current study that only focuses on analyzing marsh frog calls, any ambiguous calls that we could not reliably confirm were those of marsh frogs were excluded from data analysis.

Call Type Classification

During the field recording sessions, several call types were classified on a qualitative basis. Specifically, most of our qualitative call type classification was based on the onomatopoeic qualities of the calls: for example, the chirping call resembles chirping sounds emitted by birds and microbats, and the word “woah” resembles the way the “woah” calls sound. However, we decided to use the term “laughing calls”, as the calls we decided to label with the term are similar to human laughing sounds. After qualitative classification, the call types were confirmed and refined based on the field recordings and spectrograms gained from the field sessions.

Acoustic Parameter Extraction

To perform statistical analyses on the acoustic characteristics of the call units of each subjectively classified call type, the acoustic parameters of the call units were extracted using

² Prior to the study, I underwent intensive ear training by listening to recordings of marsh frog, pool frog, and edible frog vocalizations found on websites such as AmphibiaWeb and national park websites and YouTube.

Avisoft SASLab Lite (Filiciotto et al., 2014). Before extracting the acoustic parameters of the vocalizations, the left microphone input of all the three recordings were removed, as the left microphone input contained acoustical information identical to the right microphone input. Sections of the recordings where there were few or no vocalizations present were then manually removed. The remaining recordings were then manually divided into five-minute segments.

After the preparatory data processing steps, the Automatic Parameter Measurements setup in Avisoft SASLab Lite was configured to measure the temporal and structural characteristics for each call unit inspired by Hending et al. (2017), Mang'are et al. (2019), and Bright (2008) (see **Table 1**). The threshold for separating call units (i.e., the element separation threshold) was set as -20 dB. Depending on the call type being analyzed, a 600 Hz to 1200 Hz high-pass and a 1700 Hz to 5000 Hz low-pass frequency filter were applied to the recordings to ensure the call units were only analyzed based on their fundamental call units³. Then, for all but one of the call types, a noise reduction filter between -60 dB and -35 dB was applied to increase the software's accuracy in call unit identification.

³ The harmonic of a call unit with the largest amplitude.

Acoustic Parameter	Measurement unit
Duration (s)	Time between the onset and offset of a call unit
Interval (s)	Time between the offset of one unit and the onset of the next unit
Distance from start to maximum frequency (s)	Time between the onset of the unit and the unit's point of maximum frequency
Start peak frequency (Hz)	Frequency at maximum amplitude of the unit start
Start minimum frequency (Hz)	Minimum frequency at the onset of the unit
Start maximum frequency (Hz)	Maximum frequency at the onset of the unit
Start bandwidth ⁴ (Hz)	Range between maximum and minimum start frequency
End peak frequency (Hz)	Frequency at maximum amplitude of the unit end
End minimum frequency (Hz)	Minimum frequency at the offset of the unit
End maximum frequency (Hz)	Maximum frequency at the offset of the unit
End bandwidth (Hz)	Range between maximum and minimum end frequency
Mean peak frequency (Hz)	Mean frequency at maximum amplitude during the total duration of the unit
Mean minimum frequency (Hz)	Mean minimum frequency during the total duration of the unit
Mean maximum frequency (Hz)	Mean maximum frequency during the total duration of the unit
Mean bandwidth (Hz)	Range between the mean minimum and mean maximum frequencies for the total unit duration

Table 1: A list of acoustic parameters analyzed in the current study.

⁴ Range between minimum and maximum frequency at a given location within a call unit.

Data Analysis

To check the accuracy of the subjective classifications statistically, principal component analysis (PCA) and discriminant function analysis (DFA) were performed on the data. Firstly, twenty to 21 call units were selected for data analysis in each call type. The extracted parameters were manually transcribed into a Microsoft Excel worksheet in which a column was dedicated to each of the variables. The worksheet also contained the qualitatively classified call type of each call unit, as well as the name of the five-minute condensed recording from which a call unit was extracted consisting of the name of the raw recording from which the five-minute condensed recording originated and a time range indicating where in the full condensed recording the five-minute condensed recording was located. Means and standard deviations of the frequencies at the maximum amplitude of the location of maximum amplitude were calculated for the call units within each call type, and a box-and-whiskers plot was created to visually display the distribution of the values.

To prepare the call unit data for statistical analyses in R (R Core Team, 2021) which was conducted using R Studio, another Excel table was created in which each qualitatively classified call type was assigned a numeric label and in which the call unit values for each extracted parameter except the interval parameter were logarithmically transformed with a base of ten. The logarithmic transformation was conducted because the magnitude differences between the frequency- and time-related values would have otherwise exceeded the assumptions of the PCA and DFA analyses. As not all call types had interval values (i.e., the call units in some call types occurred individually), the interval parameter was removed from the new table and excluded from statistical analyses.

An approach following Hending et al. (2022) was adopted to perform PCA on the extracted acoustic dataset, where accuracy of the qualitative call type classification was determined through re-classifying of the existing call types based on their principal components, combinations of the initial variables that explain to the greatest degree the variance of all the variables (Greenacre et al., 2022). DFA was subsequently performed, where the qualitatively classified call types were reclassified based on the extracted parameters of their acoustic variables in order to determine the accuracy of the subjectively determined marsh frog vocal repertoire.

Qualitative Observations

In addition to quantitatively determining the call types of marsh frogs, qualitative observations focusing on the behaviors of the frogs whilst they were emitting certain calls and orders in which some call types often occurred together were also noted in a field notebook. The notebook contained detailed information about patterns in the progression of vocal choruses as a whole, as well as occasional changes in the environmental context, such as the sudden occurrence of anthropogenic noises, during the raw recordings.

RESULTS

Quantitative Results

A total of eight call types – laughing calls, chirping calls, normal high-frequency descending “woah” calls, disrupted high-frequency descending “woah” calls, medium-frequency ascending “woah” calls, rough low-frequency ascending “woah” calls, fine low-frequency ascending “woah” calls, and “breck” calls – were subjectively identified. The spectrograms of

the eight call types before filtering are displayed in **Figure 3**. The numerical labels assigned to the call types and the raw recordings from which the call units in each call type were selected for data analysis are displayed in **Appendix C**.

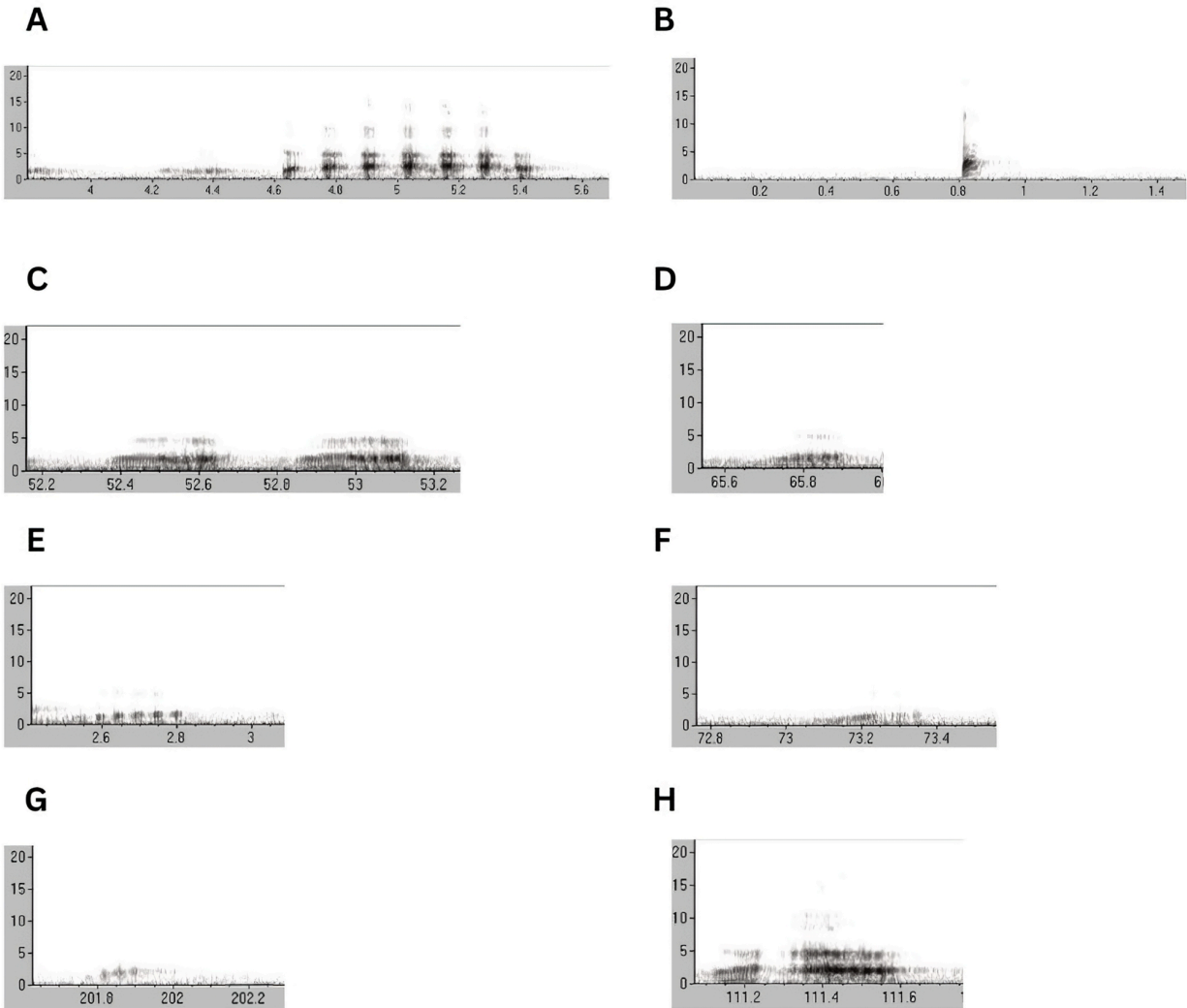


Figure 3: Spectrograms of the eight observed marsh frog call types without filtering: (A) a series of seven laughing call units; (B) a chirping call; (C) two normal high-frequency descending “woah” calls; (D) a medium-frequency ascending “woah” call; (E) a series of six rough low-frequency ascending “woah” call units; (F) a fine low-frequency ascending “woah” call; (G) a “breck” call; and (H) a disrupted high-frequency descending “woah” call. Values on the x-axis represent time in s, and values on the y-axis represent frequency in kHz.

The means and standard deviations of the measured acoustics variables for each call type are listed in **Table 2**. According to the table, the call type with the highest mean peak frequency of its call units is the chirping call (mean = 2440 Hz, $SD = 486.015$ Hz), and the call type with the lowest mean peak frequency is the rough low-frequency ascending “woah” call (mean = 1166.667 Hz, $SD = 228.765$ Hz). The call type with the longest duration of its call units is the disrupted high-frequency descending “woah” call (mean = 0.346 s, $SD = 0.067$ s), and the call type with the shortest duration of its call units is the laughing call (mean = 0.015 s, $SD = 0.008$ s). Furthermore, the multi-unit call type with the largest interval between its call units is the normal high-frequency descending “woah” call (mean = 0.471, $SD = 0.086$), and the multi-unit call type with the smallest interval between its units is the laughing call (mean = 0.060, $SD = 0.011$).

Call type	Laughing		Chirping		Normal high-freq. descending "woah"		Medium-freq. ascending "woah"		Rough low-freq. ascending "woah"		Fine low-freq. ascending "woah"		Breck		Disrupted high-freq. descending "woah"	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Duration (s)	0.015	0.008	0.020	0.009	0.249	0.047	0.167	0.046	0.018	0.007	0.256	0.095	0.124	0.029	0.346	0.067
Interval (s)	0.060	0.011	N/A	N/A	0.471	0.086	N/A	N/A	0.063	0.049	N/A	N/A	N/A	N/A	N/A	N/A
Dist. from start to max. freq. (s)	0.007	0.006	0.006	0.005	0.200	0.051	0.109	0.033	0.006	0.007	0.169	0.057	0.062	0.024	0.219	0.066
Start peak freq. (Hz)	1985.000	328.113	2120.000	505.340	1875.000	259.301	905.000	198.614	1133.333	195.789	828.571	130.931	930.000	205.452	1640.000	357.477
Start min. freq. (Hz)	520.000	478.594	980.000	626.267	1090.000	350.789	310.000	137.267	528.571	219.415	252.381	87.287	295.000	146.808	840.000	104.630
Start max. freq. (Hz)	4230.000	106.1330	4465.000	257.1355	2975.000	410.150	2000.000	343.358	2076.190	214.254	1933.333	217.562	1980.000	294.868	3015.000	352.846
Start bandwidth (Hz)	3635.000	111.2737	3435.000	289.6872	1835.000	468.227	1660.000	348.531	1519.048	180.607	1647.619	244.170	1650.000	326.867	2100.000	346.410
End peak freq. (Hz)	2045.000	356.112	2335.000	533.385	1905.000	139.454	1560.000	245.807	1180.952	246.209	1190.476	260.585	1280.000	219.089	1735.000	367.459
End min. freq. (Hz)	585.000	464.843	1430.000	773.985	1180.000	289.464	625.000	312.671	471.429	232.686	347.619	163.153	445.000	258.488	840.000	201.050
End max. freq. (Hz)	4360.000	127.8321	3685.000	848.699	2875.000	158.529	2630.000	222.663	2180.952	222.753	2219.048	166.190	2390.000	255.260	3115.000	378.744
End bandwidth (Hz)	3710.000	155.2214	2210.000	134.2778	1620.000	369.352	1956.000	429.178	1661.905	233.401	1800.000	167.332	1890.000	398.550	2220.000	418.770
Mean peak freq. (Hz)	2005.000	281.864	2440.000	486.015	1815.000	103.999	1435.000	181.442	1166.667	228.765	1185.714	101.419	1295.000	143.178	1755.000	310.305
Mean min. freq. (Hz)	1400.000	385.255	1730.000	589.469	1165.000	200.722	680.000	260.768	519.048	191.361	500.000	122.474	575.000	120.852	940.000	308.477
Mean max. freq. (Hz)	3020.000	444.380	3380.000	502.206	2860.000	153.554	2505.000	170.062	2114.286	239.344	2104.762	135.927	2260.000	142.902	2885.000	434.408
Mean bandwidth (Hz)	1535.000	703.581	1600.000	526.158	1625.000	214.905	1805.000	218.789	1557.143	246.113	1595.238	149.921	1620.000	143.637	1875.000	495.108

Table 2: Means and standard deviations of all measured acoustic variables for each call type.

PCA in R of the eight subjectively classified call types displayed a cumulative proportion of variance of 0.65; the first two principal components were responsible for 65% of the overall variance (**Table 3**). Visualization of the first two principal components of the eight subjectively classified call types show that the call units belonging to the different call types are somewhat distinct (**Figure 4**).

	PC1	PC2	PC3	PC4	PC5
Standard deviation	2.5863	1.5554	1.2434	0.94991	0.88016
Proportion of Variance	0.4778	0.1728	0.1104	0.06445	0.05533
Cumulative Proportion	0.4778	0.6506	0.7610	0.82546	0.88080

Table 3: A table summarizing results of the first five principal components (PC) from the PCA of the eight subjectively classified call types.

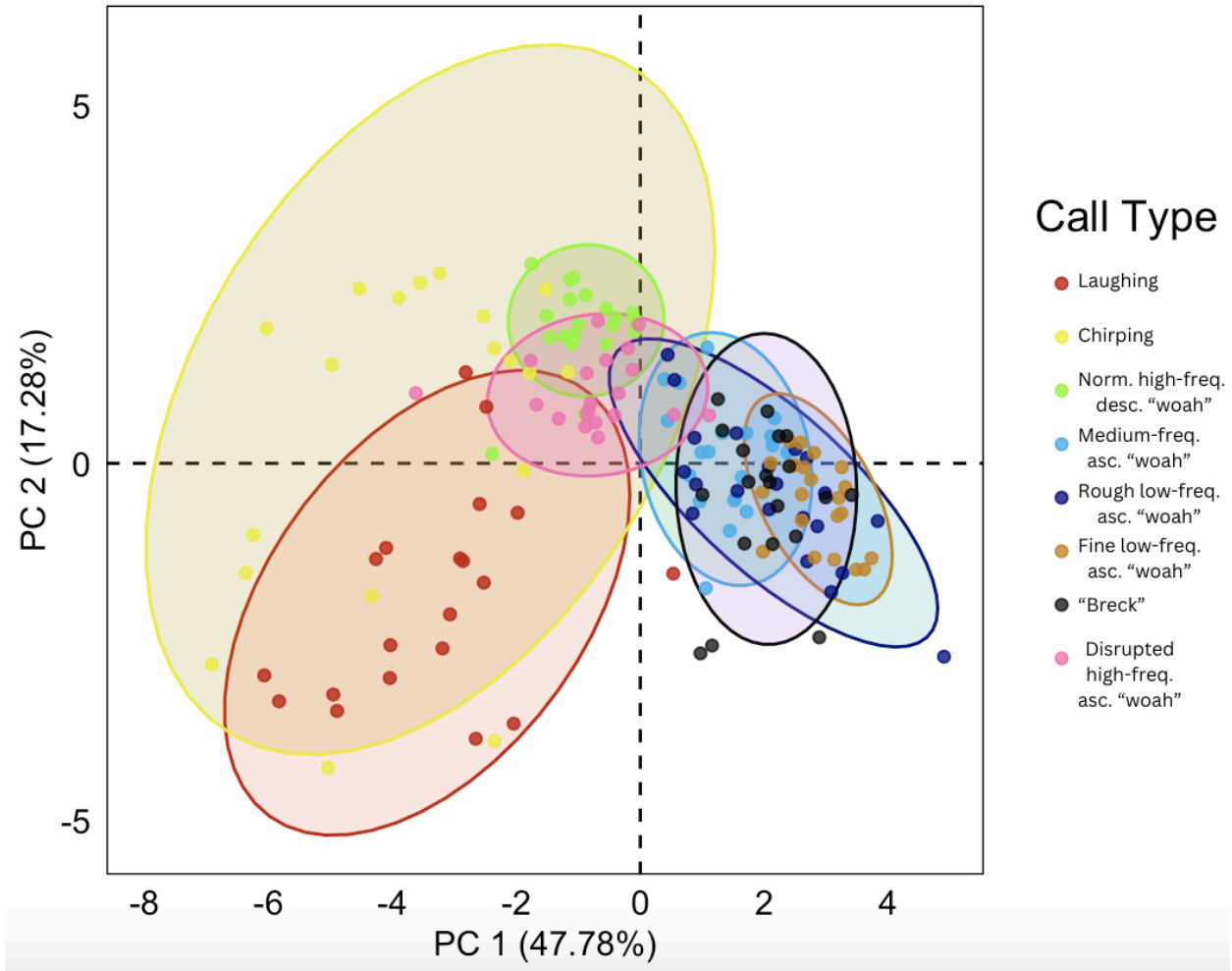


Figure 4: Visualization of the first two principal components of the eight subjectively classified call types in R. The x-axis represents the first principal component which explains 47.78% of the total variance, and the y-axis represents the second principal component which explains 17.28% of the total variance. The dots represent individual call units. Each subjectively classified call type is represented with a distinct color, and the areas occupied by call units from different call types are displayed by ellipses.

DFA showed that the call type with the largest proportion (100%) of call units correctly identified subjectively was call type 5 (i.e., the rough low-frequency ascending “woah” calls) and that the call type with the smallest proportion (65%) of call units correctly identified subjectively was call type 4 (i.e., medium-frequency ascending “woah” calls) (**Table 5**). The mean proportion of correctly identified call units within the call types was 78.96%. Results of the DFA revealed

statistically significant differences between the eight subjectively classified call types (Wilk's $\lambda = 0.388$, $F = 16.580$, $p < 0.001$). For a more detailed breakdown of the DFA outcomes, see **Table 4**.

	1	2	3	4	5	6	7	8
1	15	4	0	0	1	0	0	0
2	5	15	0	0	0	0	0	0
3	0	0	18	0	0	0	0	2
4	0	0	0	13	0	1	6	0
5	0	0	0	0	21	0	0	0
6	0	0	0	3	0	14	3	1
7	0	0	0	4	0	2	14	0
8	0	0	2	0	0	0	0	18

Table 4: DFA results displayed as a table in R. Numbers in the first row and first column represent the numerical labels assigned for each subjectively classified call type (**Appendix C**). The table should be read horizontally – values in each row excluding the call type numbers should add up to the number of call units analyzed for the given call type. For example, for call type 2 (i.e., the chirping calls), 15 of the 20 call units analyzed were correctly identified subjectively through DFA as call type 2, and five of the 20 call units analyzed were incorrectly identified as call type 1. The cells containing the number of correctly identified call units for each call type according to the analysis are highlighted in green.

Call type number	1	2	3	4	5	6	7	8
Proportion of call units correctly identified	0.75	0.75	0.90	0.65	1.00	0.67	0.70	0.90

Table 5: The proportion of correctly identified call units within each subjectively classified call type in DFA. The top row displays the numerical values assigned for the call types, and the bottom row displays the proportion values. For example, 90% of call units in call type 3 were correctly identified subjectively as call type 3.

Qualitative Results

Several frequent vocalization mechanisms and behaviors were observed during the field recordings. The laughing calls were observed to be emitted by the frog expanding its side vocal sacs and minimally shaking them for a relatively longer duration. Chirping calls⁵ were observed to be followed by a physical displacement of the frog. High-frequency descending “woah” calls were accompanied by the rapid expansion and contraction of the side vocal sacs. However, lower-frequency “woah” calls were accompanied by the rapid inflation of the central vocal sac and minimal inflation of the side vocal sacs.

Three frequently occurring call sequences were noted as part of the qualitative observations. First, laughing call sequences consisting of groups of laughing call units followed by similar laughing call unit groups with gradually increasing numbers of call units were often observed. Another frequent call sequence started with a medium-frequency ascending “woah” call or a hybrid call with ascending intonation, followed by high-frequency descending “woah” calls, and ending with a fine or rough low-frequency ascending “woah” call. Finally, medium-frequency ascending “woah” calls and mixed “breck” and medium-frequency ascending “woah” calls were also observed to occur one after the other, followed by a low-frequency ascending “woah” call. Some trends in vocalizations, such as the overlapping of “woah” and laughing calls, co-occurrence of multiple disrupted high-frequency “woah” calls, and the mixing or gradual transitioning between the ascending “woah” call types, the “breck” calls and medium-frequency ascending “woah” calls, and the laughing and “woah” calls, were also observed.

⁵ I observed from YouTube videos of vocalizing marsh frogs recorded in other geographical areas that marsh frogs emitted chirping calls using their mouth instead of their vocal sacs. The exact physical mechanism by which chirping calls are emitted remains to be studied.

DISCUSSION

Quantitative classification

The present study subjectively identified a total of eight call types emitted by marsh frogs in the Margaret Island pond based on field recordings and observations. While DFA suggested that the subjectively identified call types were different from each other at the highest level of statistical significance, PCA of the call types yielded results of borderline statistical significance with a cumulative proportion of variance of 0.65 of the first two principal components⁶. This result may be due to the small number of call units used in this study for data analysis; increasing the number of call units used for data analysis will likely increase the cumulative proportion of variance of the first two principal components. It is also possible that with an increased number of call units analyzed, the overlaps between the areas occupied by the call types in the PCA plot become smaller.

The laughing, chirping, high-frequency descending “woah”, medium-frequency ascending “woah”, and fine low-frequency ascending “woah” calls identified in this study are similar to the “mating”, “warning”, “territorial”, “release”, and “aggressive” calls described in Lukanov et al. (2011), respectively. However, this study identified three additional call types, the rough low-frequency ascending “woah” calls, “breck” calls, and disrupted high-frequency descending “woah” calls, that were not present in Lukanov et al. (2011). Lukanov et al. (2011) also described a call (the “distress” call) that was not present in the recordings used in the current study. These differences in the described vocal repertoire of marsh frogs may be due to the recordings used in the two studies capturing some but not other call types or geographic differences in marsh frog vocalizations.

⁶ In PCA, a cumulative proportion of variance of the first two principal components of above 0.70 is generally considered statistically significant for bioacoustic call type analyses.

Qualitative observations

Qualitative observations documented some behavioral correlates related to certain marsh frog vocalizations, as well as placed some of the identified call types into wider contexts. Firstly, the rapid displacement often observed following the emission of the chirping call suggests that certain calls can encode specific information and behaviors, highlighting the importance of categorizing call types based on functional correlates (Toledo et al., 2015). Additionally, the frequent occurrence of specific call sequences may indicate that combining multiple calls may enhance or vary the meaning of the individual calls. According to Bhat et al. (2022), some frogs are capable of changing their vocal sequences based on contextual information, but whether and how marsh frogs adjust their call sequences remains to be investigated. Furthermore, the occurrence of hybrid calls and frequent gradual transitioning between calls support the observation of Kuhn and Schneider (1984), according to which marsh frogs emit transitional calls with intermediate features. Combined with the findings of Kuhn and Schneider (1984), this result implies that hybrid and transitional marsh frog calls are present in multiple geographic regions. Finally, after comparing the observed behaviors associated with the emission of medium-frequency ascending “woah” calls to the naming of the “distress” calls described in Lukanov et al. (2011), we also raised questions related to marsh frogs’ potential use of the same or similar call types for different social functions in different geographic areas. Such questions may inspire further research in the area of marsh frog communication with a focus on behavioral ecology.

Moreover, when combined with the quantitative findings, qualitative results of the current study may also shed light on the assumption that marsh frogs differentiate between the functions or contexts of their calls based on the acoustic structure of the calls. This assumption complies

with the finding of Yue et al. (2017), according to which frogs tend to attend to a specific portion of calls emitted by conspecifics more due to the larger amount of information it seems to carry. The assumption also aligns with the suggestion of Bevier et al. (2004), according to which specific frog calls have the function of mediating social interactions and spatial relationships. As eight to 13 marsh frogs were spotted in the same pond during the making of the recordings analyzed in the current study, which occurred amidst their mating season, it is possible that the identified call types played an important role in the modulation of social interactions in this population.

Limitations

Although the current study addressed several gaps identified in Lukanov et al. (2011), there are some limitations which must be acknowledged and considered for future studies. First, only 20 call units per call type were selected for data analysis in the present study. The small amount of data used may have limited the statistical power of our analyses. Despite having been present in several recordings, a few ambiguous calls, the acoustic characteristics of which resembled those of edible frog vocalizations, were omitted from data analysis. Although those omitted calls may in fact have been emitted by edible frogs, there is a possibility of those calls belonging to new call types emitted by marsh frogs. Furthermore, the background noise and the long distance between the frogs and the recording device during the recording sessions may have resulted in lower quality spectrograms for a small number of the call units, decreasing the accuracy of call type classifications. Finally, to conduct qualitative observations at night during the study period, a torch was used to detect individual marsh frogs and observe their vocalization mechanisms and behaviors. The powerful light emitted by the torch may have influenced marsh

frogs' vocalization behavior by potentially causing them to avoid emitting specific calls or to abnormally emit certain other calls.

Implications for Conservation and Future Research

The current study has a number of implications for future research not only in the areas of amphibian vocal communication and vocalization-behavior matching but also in the areas of urban bioacoustics and the design of amphibian conservation technology. The qualitative results in the study demonstrated the need to design controlled experimental studies to further investigate potential specific behaviors associated with each call type as well as the exact physical mechanisms by which marsh frogs in this geographic region emit different types of vocalizations. Also, studying the environmental and communicative contexts in which certain frequently occurring call sequences are emitted may have implications for our understanding of amphibian cognition and communication, potentially leading to the detection of a marsh frog “language” or several marsh frog “dialects” (Schneider & Sofianidou, 1985; Lukanov et al., 2014). Moreover, the marsh frog vocalizations identified in this exploratory study may be compared with marsh frog vocalizations in areas less inhabited by humans, allowing future researchers to use the results of the current study as a starting point for the investigation of potential effects of anthropogenic noise on marsh frog acoustic communication in central Europe (Lukanov et al., 2014; Lukanov et al., 2014). From an engineering perspective, characterizing the identified call types in greater detail may also help in the design of mechanical devices used for the estimation of the size of marsh frog populations and marsh frog chorusing activity at a given site based on total acoustic energy (Marques et al., 2012; Xie et al., 2016; Simmons et al., 2022). Moreover, assisting conservation efforts, results of the study may inspire the design of technology used for the detection and identification of marsh frogs based on their acoustic

features within or beyond the borders of Hungary (Brodie et al., 2020; Ting Yuan & Athiar Ramli, 2013; LeBien et al., 2020; Lapp et al., 2021).

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STATEMENTS AND DECLARATIONS

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Appendix A: A table listing the name, date, start time, length, and weather-related information of the recordings used in the present study

Name of recording	Date (with start time)	Length in hr:min:s	Air temp.	Water temp.	Air humidity	Weather	Air Quality Index (AQI)
Recording1	May 27, 2022 (8:48 pm CEST)	1:02:24	22°C	22°C	52%	Partly cloudy	29 (good)
Recording2	May 28, 2022 (9:09 pm CEST)	1:00:24	16°C	19°C	41%	Mainly cloudy	32 (good)
Recording3Part1	May 28, 2022 (10:26 pm CEST)	0:35:30	14°C	14°C	45%	Cloudy	32 (good)

Appendix B: Oscillograms and spectrograms of the marsh frog call types identified by Lukanov et al. (2011) with original descriptions

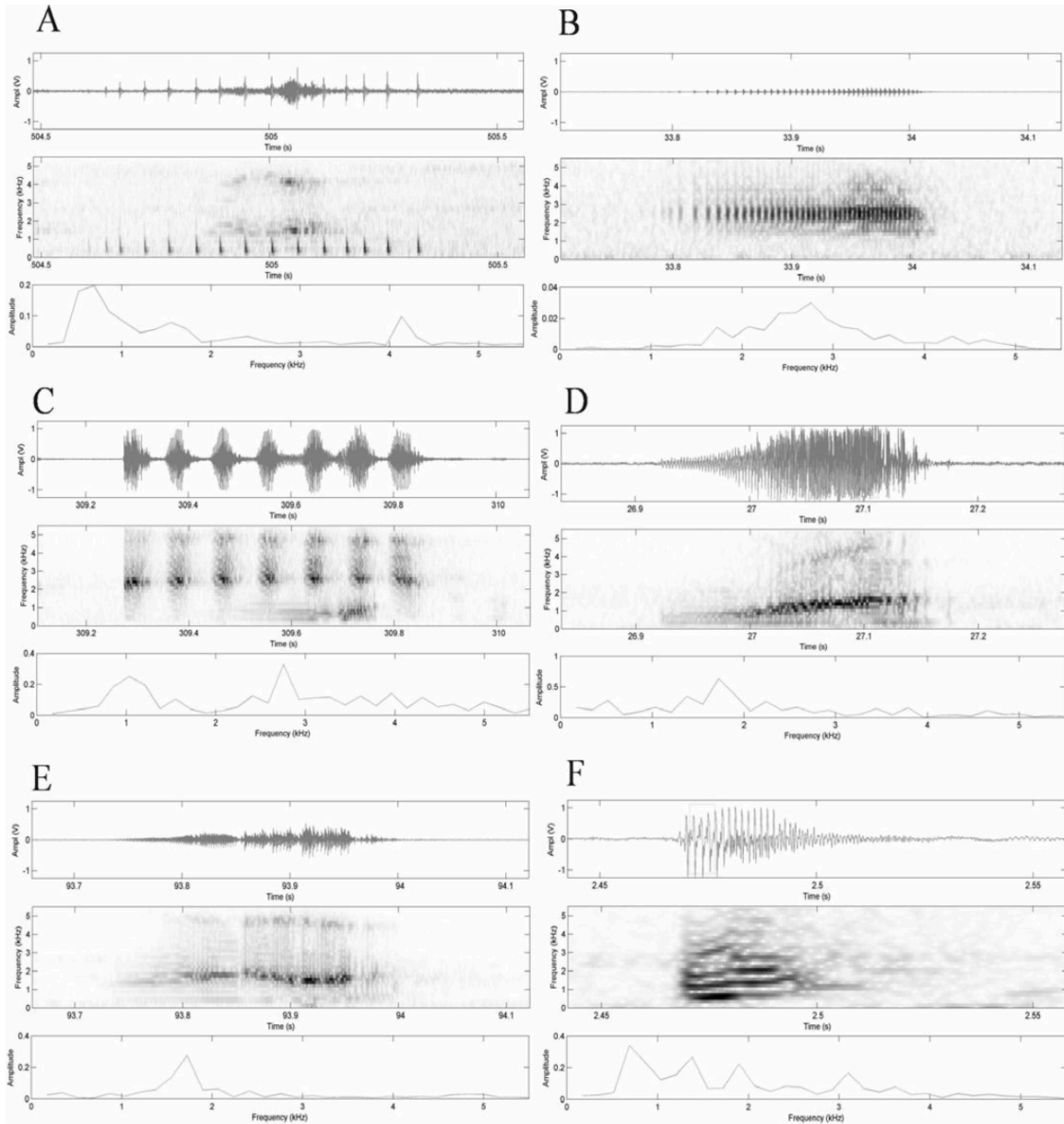


Fig. 1: Oscillograms and spectrograms of call types encountered in *Pelophylax ridibundus*: aggressive call (A), distress call (B), mating call (C), release call (D), territorial call (E) and warning call (F)

**Appendix C: A table summarizing the raw recordings needed for the analysis of call units
in each call type**

Name of call type	Assigned numerical label for call type	Recordings used for analyzing the call units
Laughing call	1	Recording1
Chirping call	2	Recording1, Recording2, Recording3Part1
Normal high-frequency descending "woah" call	3	Recording1
Medium-frequency ascending "woah" call	4	Recording1
Rough low-frequency ascending "woah" call	5	Recording1
Fine low-frequency ascending "woah" call	6	Recording1
"Breck" call	7	Recording1
Disrupted high-frequency ascending "woah" call	8	Recording1, Recording2, Recording3Part1