

Effect of anthropogenic noise on call parameters of *Hyla arborea* (Anura: Hylidae)

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Abstract. Increasing urbanisation in recent decades has subjected many species and their populations to the influence of anthropogenic noise generated by human technologies. Noise pollution can negatively affect acoustic communication in representatives of different taxa and frogs, as the most vocally active amphibians, are especially vulnerable to such adverse effects. In the present study, we tested the hypothesis that anthropogenic noise could alter the mating call parameters in the European treefrog. The two chosen study sites were in the same geographic area and had similar characteristics except for the presence of anthropogenic noise source. Recordings were made in a WAV-PCM format and both spectral and temporal call parameters were analysed using specialised software. Results demonstrated significant differences in most of the studied parameters, suggesting that treefrogs are able to alter their calls and communicate successfully in moderate levels of anthropogenic noise.

Keywords: amplitude, European treefrog, frequency, mating call, noise pollution.

1. Introduction

The only representatives of the widespread family Hylidae on the European continent are the members of the *Hyla arborea* species complex. The European treefrog, *Hyla arborea* (L., 1758) is distributed from the Southern Balkans to North-Western Europe (Speybroeck et al., 2016). It was recently established that most treefrog populations on the territory of Bulgaria, previously thought to be *H. arborea*, belong to its sister taxon *Hyla orientalis* Bedriaga, 1890 and according to molecular data, the former is only present in the Struma river basin (Dufresnes et al., 2015).

Anurans are the most vocally active amphibians and sound plays a key role in their communication. Treefrogs make no exception to that rule, with some choruses reaching sound pressure levels of 110 dB (Bee, 2012). Abiotic background noise is a major hindrance to successful com-

munication, as it decreases signal to noise ratio and reduces the chances the signal will be received or properly recognised (Lengagne, 2008). Some studies have also demonstrated that anthropogenic noise alters mate preferences in females (Bee & Swanson, 2007). Increasing urbanisation in recent decades has meant that noises generated by human technologies are ever more likely to interfere with wildlife acoustic communication.

There has been a decline in treefrog populations across Europe for the past decades, and it is generally agreed that the increasing density of urban areas and proximity to roads have a negative effect on the presence of calling males (Borgula, 1993; Pellet et al., 2004). The overall negative effects of roads on amphibians and reptiles have been studied in many publications (for review see Jochimsen et al., 2004) but the impact of anthropogenic noise is still poorly understood. There are two main hypotheses

explaining how species can cope with auditory masking in the presence of anthropogenic noise: the Lombard effect that suggests individuals emit louder calls (Junqua et al., 1999) and the acoustic adaptation hypothesis that suggests individuals shift their frequencies out of the noise range (Morton, 1975). However, these hypotheses are based on birds and little evidence to support them has been found in anurans (for review see Roca et al., 2016). Behavioural responses to anthropogenic background noise in frogs can include avoidance of noise source, decreased mobility and chorus tenure or modification of spectral and/or temporal call parameters (Nash et al., 1970; Fahrig et al., 1995; Slabbekoorn & Peet, 2003; Kaiser et al., 2011; Lukanov et al., 2014).

In the present study, we test whether parameters of the mating call of the European treefrog are affected by anthropogenic noise produced by a gas compressor station that has been operating for more than 10 years. We hypothesize that both spectral and temporal parameters of calls from this site should differ to those of calls from a site without a constant presence of abiotic noise.

2. Materials and methods

2.1 Study sites

Two microdams from the Struma valley in South-western Bulgaria were visited for the purpose of the study. They were designated as “noisy” (site 1) and “quiet” (site 2) based on the ambient level of anthropogenic noise. In site 1 (N 41.45499°, E 23.24301°) the noise was caused by a gas compressor station located next to the microdam; in site 2 (N 41.45594°, E 23.37475°) there were no sources of anthropogenic noise. Both microdams were of similar size and depth (i.e., 50 m in diameter and maximum depth of above 2 m), with shallow banks overgrown with reed. The distance between the two sites on a straight line is about 11 km, with site 1 to the West and site 2 to the East. In site 1 the nearest building (the gas compressor station) is within 25 m of the eastern bank of the microdam, with the village of Rupite being about 1km to the South; in site 2 the nearest building (a house) is about 500m to the East, in the village of Harsovo (a road connecting the villages of Harsovo and Kromidovo is 50m to the North of site 2 – however, it is rarely used by vehicles, so it did not influence ambient noise levels). Both sites are surrounded by meadows and crop fields, with few shrub vegetation and trees in a 200-meter radius.

2.2 Sound recording and processing

Ambient noise levels were measured using a noise meter with C-weighting. C-weighting was preferred over A-weighting as the latter cuts off frequencies not perceived by the human ear (i.e., from the higher and lower spectrum) (Ladefoged, 2001).

Treefrog mating calls were recorded using an Olympus LS-5 linear recorder in a WAV-PCM mode with sampling frequency 44.1 kHz, 20-21,000 Hz and 24-bit resolution. Recordings were made during breeding season (March-May) 2018 with water and air temperature between 19-20°C and 20-22°C, respectively. Only individual calls and not choruses were recorded. All recorded individuals were adult males with similar body size of 40-50 mm in order to prevent frequency differences linked to body size. Recordings with lesser quality (e.g., strong background noise, overlapping calls, etc.) were not selected for the analyses. The rest were processed with the computer program Soundruler v. 0.9.6.0. (Gridi-Papp et al., 2007). A total of 100 individual mating calls (50 from each study site) were measured. The following call parameters were used for the analyses: 1. Call duration (CD); 2. Number of pulses per call (PN); 3. Duration of pulses (PD); 4. Duration of interval between pulses (ID); 5. Number of notes per pulse (NN); 6. Call dominant frequency (DF); 7. Ratio of peak frequency/bandwidth at 50% peak amplitude (Tune50); 8. Ratio of peak frequency/bandwidth at 10% peak amplitude (Tune10); 8. Relative amplitude of the first harmonic (RA1); 10. Relative amplitude of the third harmonic (RA3). Spectral parameters (DF, Tune50, Tune10, RA1, RA3) were processed at a FFT (Fast Fourier Transformation) length of 1024 points; temporal parameters (CD, PN, PD, ID, NN) were measured in seconds in the oscillograms. All measurements were verified manually as recommended by Bee (2004). A coefficient of variation between calls ($CV\% = SD/mean \times 100$) was calculated in order to differentiate between static ($CV\% < 5$) and dynamic ($CV\% > 12$) call parameters (Gerhardt, 1991).

2.3 Data analysis

All data was tested for normality with the Shapiro-Wilk test and the null hypothesis was rejected ($p=0.000$). To avoid log-transformation, a Mann-Whitney U test was performed for the whole dataset of call parameters with Study site as a grouping variable. Test for difference between two proportions was used to compare CV% for each call parameter between the two study sites. All tests were carried out using the STATISTICA v.7.0 software (StatSoft, Inc., 2004) and the chosen alpha level for statistical significance was $p < 0.05$.

3. Results

Ambient noise levels were 41-76 dB (55 ± 17.6) in site 1 and below 30 dB in site 2. The spectrum of the noise

from the compressor station covered the range 97-1776 Hz, partially overlapping the frequency of the treefrog mating call (Fig.1).

Descriptive values for studied call parameters are presented in Table 1.

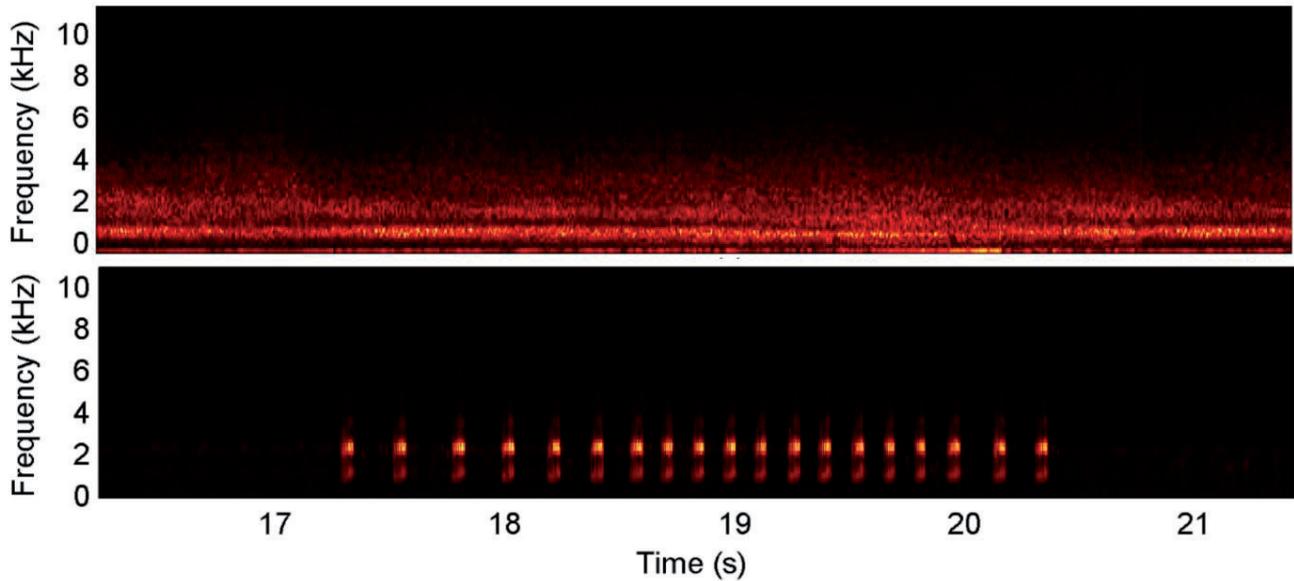


Figure 1. Spectrograms of noise (above) and a mating call of *Hyla arborea* from site 1 (below; the 19 pulses and their harmonic structure are clearly visible)

Table 1. Values of the studied call parameters

	site 1				site 2			
	Min	Max	Mean \pm SD	CV%	Min	Max	Mean \pm SD	CV%
CD	1.950	3.839	2.643 ± 0.570	21.6	1.887	5.313	3.405 ± 0.896	26.3
PN	10	29	18.778 ± 6.091	32.4	13	28	19.405 ± 3.797	19.6
PD	0.055	0.086	0.068 ± 0.009	13.3	0.048	0.110	0.062 ± 0.013	20.3
ID	0.089	0.194	0.137 ± 0.026	18.9	0.127	0.204	0.162 ± 0.018	11.3
NN	7	8	7.556 ± 0.506	6.7	7	9	7.514 ± 0.607	8.1
DF	1905.7	2357.9	2141.9 ± 87.1	4.1	2095.2	2421.8	2280.9 ± 73.9	3.2
Tune50	8.924	57.697	32.101 ± 8.534	26.6	3.579	27.909	14.590 ± 4.775	32.7
Tune10	2.417	27.456	7.243 ± 4.349	60.0	1.139	14.568	4.105 ± 1.051	25.6
RA1	-33.157	-3.6314	-13.510 ± 5.093	37.7	-19.268	-6.820	-13.372 ± 1.534	11.5
RA3	-37.3059	-16.7857	-27.069 ± 3.958	14.6	-35.480	-20.622	-26.007 ± 1.755	6.7

Notation: CD – Call duration; PN – Number of pulses per call; PD – Duration of pulses; ID – Duration of interval between pulses; NN – Number of notes per pulse; DF – Call dominant frequency; Tune50 – Ratio of peak frequency/bandwidth at 50% peak amplitude; Tune10 – Ratio of peak frequency/bandwidth at 10% peak amplitude; RA1 – Relative amplitude of the first harmonic; RA3 – Relative amplitude of the third harmonic.

There were no differences between both study sites in terms of the CV%, with all parameters except DF falling into the “dynamic” category (Table 1). However, when comparing CV% between parameters, there were significant differences between Tune10 ($p=0.03$) and RA1 ($p=0.013$), with both parameters having considerably larger CV% in site 1. Results from the Mann-Whitney U test revealed statistically significant differences in three of the temporal parameters and all of the spectral parameters of the mating calls (Table 2).

Table 2. Mann-Whitney U test results with Study site as a grouping variable. Significant results are marked with *. For the abbreviations, see Materials and methods

	U	Z	p
CD*	282.5	2.943	0.003
PN	402	1.331	0.183
PD*	293.5	-2.794	0.005
ID*	207	3.969	0.000
NN	466.5	-0.503	0.615
DF*	87506	-24.836	0.000
Tune50*	34867.5	29.486	0.000
Tune10*	153321	18.432	0.000
RA1*	323370	2.565	0.010
RA3*	286740.5	-5.983	0.000

When comparing calls from both study sites, parameters CD, PD and ID had shorter duration for site 1 compared to site 2, DF was lower for site 1 and the parameters related to amplitude (Tune50, Tune 10, RA1, RA2) had lower values for site 2.

3. Discussion

Overall, our study indicates that anthropogenic noise can affect both temporal and spectral parameters of the mating call in the European treefrog. When discussing the results, several points have to be taken into consideration. In their analysis of mating call variation in water frogs (genus *Pelophylax*) Schneider & Sinsch (1992, 1999) outlined four call parameters that were related to temperature: duration of pulse groups, interval between pulse groups, period of pulse groups and pulse groups/s. Schneider (1999) added call duration, intercall interval and period of calls to that list (in the previous two studies, these were listed as temperature-independent). The author explains this apparent discrepancy with the differences

between univariate vs multivariate analyses. However, neither Schneider & Sinsch (1992, 1999) nor Schneider (1999) state whether data has been tested for normality prior to applying their analyses. In addition, Schneider & Sinsch (1992) acknowledge slight variations in some data sets, caused by temperature dependence in temperature-independent parameters and vice versa. The correlation coefficient (r) in Schneider (1999) was relatively weak across all temperature-dependent parameters (absolute value between 0.42 and 0.64) and while period of pulse groups had the highest absolute value of -0.73, this could be expected, considering that this parameter is the direct sum of pulse group duration ($r=-0.59$) and interval duration ($r=-0.62$). Moreover, the study of Schneider (1999) covers temperature ranges between 19.1°C and 29.3°C, while temperature differences between the two sites in the present study were within a couple of degrees Celsius. For these reasons, we think that the registered differences in temporal parameters are unlikely to be due to temperature differences between the study sites. While Lengagne (2008) found no evidence for calling plasticity that would allow male treefrogs to adapt their mating call structure (duration and frequency) to traffic noise, the frogs he used for his experiments were taken from a private natural reserve and consequently had never been exposed to such noise. Frog calls are considered to be innate rather than learned (Wells, 2007), so it is more likely that call parameters change gradually over time. This process is different to the dynamic adaptation of call structure that has been demonstrated for other vertebrate species (e.g., Lengagne et al., 1999; Slabbekoorn & Peet, 2003). The gas compressor station near site 1 has been operating for more than 10 years, and we suggest that during this time treefrog population has developed mechanisms that would enhance acoustic communication in these adverse conditions. Shorter duration of pulses and the interval between them (leading to shorter overall call duration) could allow for more efficient transfer of the emitted acoustic signals. This also seems in accordance with the results of Velez et al. (2013), who stated that while female *Hyla chrysoscelis* generally showed preference for longer calls, the presence of noise always reduced this preference. For the same species, Kuczynski et al. (2010) discovered that with increasing distance, the intervals between pulses were filling with background noise, degrading the signal – another possible reason for male treefrogs to shorten pulse and interval duration under years-long noise influence.

In terms of spectral parameters, calls recorded at site 1 differed significantly from those at site 2 in all studied variables. Since anthropogenic noise is mainly composed of low frequencies (Goodwin & Shriver 2011; Summers et al., 2011), one possible response of vocalizing animals would be to shift their signal toward higher frequencies.

However, this is not always the case, especially in anurans. Roca et al. (2016) conducted a meta-analysis on frequency shift in response to anthropogenic noise in birds and anurans, based on existing literature data. They established that while in birds there was an overall average increase in frequency of more than 400 Hz, in anurans the mean frequency shift did not differ significantly from zero. Publications on frequency shift in anurans reveal that the number of frogs that increased their dominant frequency is comparable with the number of those that shifted negatively or did not show any change (Lengagne, 2008; Parris et al., 2009; Cunnington & Fahrig, 2010; Hanna et al., 2014; Lukanov et al., 2014). For this reason, Roca et al. (2016) hypothesize that frogs compensate for the anthropogenic noise effect mainly with changes in calling amplitude and call rate instead of frequency shifts. This would explain the rather small (although significant) difference in dominant frequency between site 1 and site 2, as well as the accompanying differences in parameters related to the ratios between frequency and amplitude (Tune10, Tune50, RA1 and RA3). In response to Roca et al. (2016), Brumm & Bee (2016) suggest that some of the results from the reviewed studies may be due to poor measurement practices (e.g., constant background noise, another calling animal, etc.) and recommend alternative measurement methods based on power spectrum (e.g., Zollinger et al., 2012). While criticizing direct spectrogram measurements, Zollinger et al. (2012) point out that one could still obtain reliable data on “notes or syllables that always occur in immediate succession”. Since this is the case in the present study (unlike most birdsongs, treefrog calls have simple and repetitive structure), we feel that the used parameters reflect a real difference between mating calls, and are not a measurement artefact.

Gerhardt (1991) established that female treefrog mating preferences were affected by both static and dynamic call parameters, with the latter (call duration, call rate and pulse number) having a highly directional effect. This suggests that a large shift in frequency (a static parameter) is not necessary for successful orientation of female treefrogs and offers a potential explanation for the significantly larger CV% in for Tune10 and RA1 in site 1 – higher variability in terms of frequency/amplitude ratios may be beneficial in minimizing the auditory masking effect of the anthropogenic noise.

The present study sheds some light on the possible effects of long-lasting noise pollution on communication of the European treefrogs. Our results suggest that treefrogs can successfully cope with artificial noise in the range of 40-70 dB and this is expected, considering male frogs singing in choruses need to cope with high levels of natural ambient noise. However, it has been demonstrated that higher levels of anthropogenic noise have a negative effect on anuran behaviour (e.g., Brattstrom & Bondello, 1983;

Kight & Swaddle, 2011; Wale et al., 2013). Considering the increasing number of habitats affected by anthropogenic noise and the low number of studies on the impact of noise on anuran communication and behaviour, additional data is still needed in order to better understand this process and to facilitate frog conservation.

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