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COMPARISON BETWEEN WATER FROGS (*RANA ESCULENTA* COMPLEX) MATING CALLS

BY

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This research aims to compare the mating calls of the *Rana esculenta* complex species (*Rana lessonae, Rana esculenta* and *Rana ridibunda*). The mating calls were recorded in the field, during the mating season. The studied parameters of the mating calls were duration, frequencies, and relative amplitudes. These parameters were graphically represented as spectrograms and relative amplitude spectrums. Discriminant analysis proved that call duration is the most efficient mating call characteristic that can be used in the bioacoustical identification of the *Rana esculenta* complex species.

Introduction

Water frog species identification has become a difficult task, due to the intermediate characters of the hybridogenetic species - *Rana esculenta*. This species evolved as a result of a primary hybridisation between *Rana ridibunda* and *Rana lessonae*. Nowadays this species persists mostly by hybridogenesis [1, 2].

The identification of these frogs implies special techniques, such as electrophoresis, which are hardly available during field research. Another way to perform species identification is to compare morphometrical indices, but this method has a certain degree of relativity. An interesting alternative would be to identify the species after their call acoustical characteristics.

Water frog males emit different types of calls. For instance, during the reproduction period they emit territorial calls, mating calls, release call and transitional calls [6, 8, 9, 10]. The most analysed call is the matting one [9, 10, 11]. Some comparisons of certain characteristics of water frogs matting calls were not very successful [7].

The analysis of the release call of *Rana lessonae*, *Rana esculenta* and *Rana ridibunda* has been proved useful for species identification. This sound is produced when a frenetic male tries to clasp another male. The result of this acoustic action is the release of the emitting male. This particularly call is easy to record, because it can be experimentally induced. This fact would be an important detail that renders the identification more facile [12]. Yet, this method requires that captive males must be

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provoked to emit release calls, which consecutively must be recorded and analysed. All these actions are, at least, time-consuming. Thus, this fact can be a problem in surveys that requires relatively fast species identification. For this reason, mating call acoustic analysis is worthwhile.

Material and Methods

The research was carried out during the springs of the years 1999 – 2002. The mating call recordings were made in the Prut River drainage basin for *Rana ridibunda* and *Rana esculenta*, and in "Mestecănişul de la Reci" – a deciduous forest grown on a sandy area with small scattered ponds – for *Rana lessonae*. "Mestecănişul de la Reci" shelters a pure *Rana lessonae* population, which has been studied through genetic and serological techniques [3].

The analysis was carried out on 30 *Rana lessonae*, 22 *Rana esculenta* and 33 *Rana ridibunda* mating call recordings.

The recordings were made with a tape recorder (Panasonic model no. RQ-L30) with a built-in, omnidirectional, condenser microphone. The bioacoustic analysis [5] was made with specific software and consisted in the computation of spectrograms and average spectrums. Data transformation used in this analysis was performed through FFT (Fast Fourier Transformation) algorithm with sample size 64. The graphics were created using a Hanning smoothing window function. The spectrum line resolution was 172.66 Hz and the time line resolution was 0.06 s.

Values of the average amplitude spectrums were statistically analysed and an arithmetic mean of the average relative amplitudes corresponding to a certain frequency was computed for each of the three species. These arithmetic means were then graphically represented as medium average amplitude spectrums for each species.

The significance of the differences between species mating call duration was tested with ANOVA single factor test [4].

The potential of critical frequencies relative amplitudes to make a difference between species was tested through the discriminant analysis. Thus, typical specimens from each species were selected for an accurate initial identification.

Results and Discussion

Mating calls duration descriptive statistic (Table 1.) revealed that the samples were relevant because the mean and the median were almost similar.

The mean duration of the call was the different for each species – 1.31±0.099 s for *Rana lessonae*, 0.84±0.081 for *Rana esculenta* and 0.67±0.034 for *Rana ridibunda*. The above-mentioned intervals are statistically significant (alfa=0.05).

Rana lessonae has the longest call and *Rana rindibunda* has the shortest of all. From the duration point of view, *Rana esculenta* has an intermediate position.

Table 1. Descriptive statistics of *Rana lessonae* (les), *Rana esculenta* (esc) and *Rana ridibunda* (rid) mating call duration

Descriptors	les	esc	rid
Count	30	22	33
Mean	1.313787	0.845423	0.671533
Median	1.3338	0.80805	0.6706
Minimum	0.8349	0.5869	0.4605
Maximum	1.962	1.2868	0.8955
Range	1.1271	0.6999	0.435
Standard Deviation	0.265817	0.184312	0.097679
Standard Error	0.048531	0.039296	0.017004
Confidence Level(95.0%)	0.099258	0.081719	0.034635

The results of the ANOVA single factor test concerning the differences among mean duration values the mating calls were the followings: value of the statistic $F_{(2,82)}$=90.422; corresponding probability P=1.814E-21. With a significance level of 0.05, one can reject the hypothesis that there is no significant difference between the 3 samples and accept that the values of mating call duration of each species are significantly different (Figure 1.).

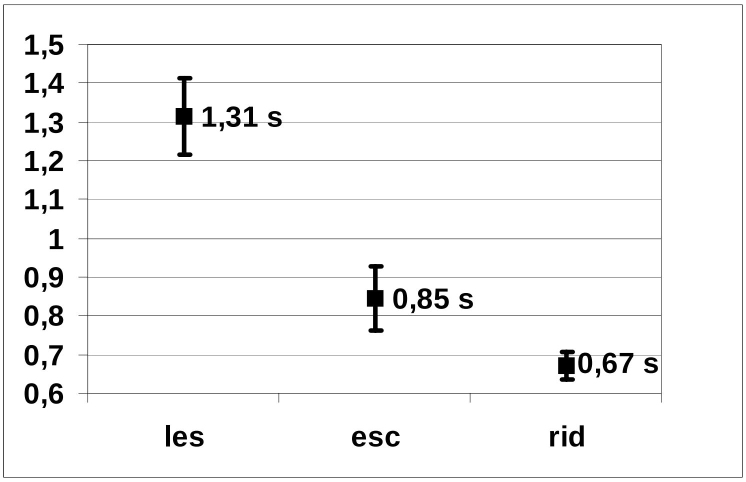

Figure 1. The result of ANOVA multiple comparison of mating call duration values (les – *Rana lessonae*, esc – *Rana esculenta*, rid – *Rana ridibunda*)

The spectrogram analysis outlines the differences between the relative amplitudes corresponding to similar frequencies. *Rana lessonae* mating call is emitted in short series of 4 – 8 sound bursts. One call includes 30 – 40 pulses with similar relative amplitudes between the frequencies of 500 – 3000 Hz (Figure 2.). *Rana esculenta* mating call comprises 13 – 15 pulse groups with the maximum amplitude energy distributed around 2000 Hz (Figure 3). *Rana ridibunda* mating call is a series of 7 – 8 pulse groups; the spectrogram (Figure 4.) shows that high amplitudes correspond to frequencies between 2000 – 3000 Hz [8, 9].

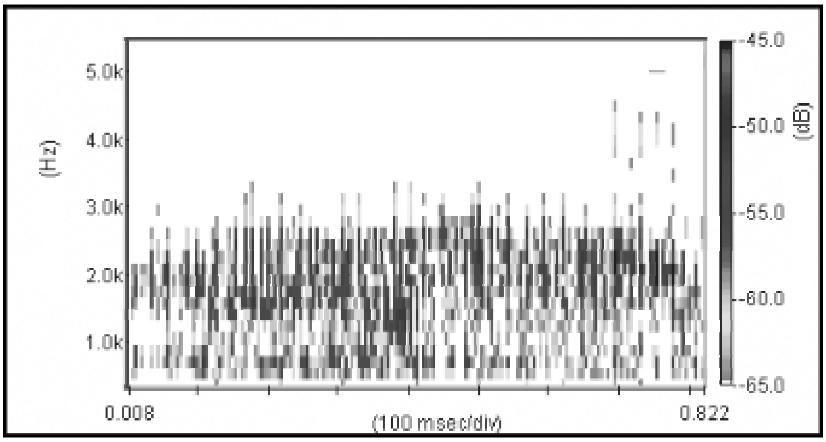

Figure 2. *Rana lessonae* mating call spectrogram

Rana esculenta call has a pulse structure that is intermediate between the other tow species. The spectrogram of *Rana esculenta* is just another aspect of the hybrid status of this species.

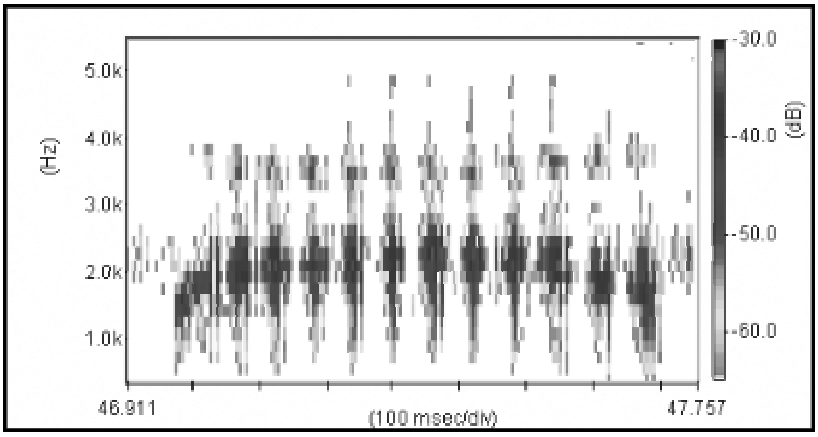

Figure 3. *Rana esculenta* mating call spectrogram

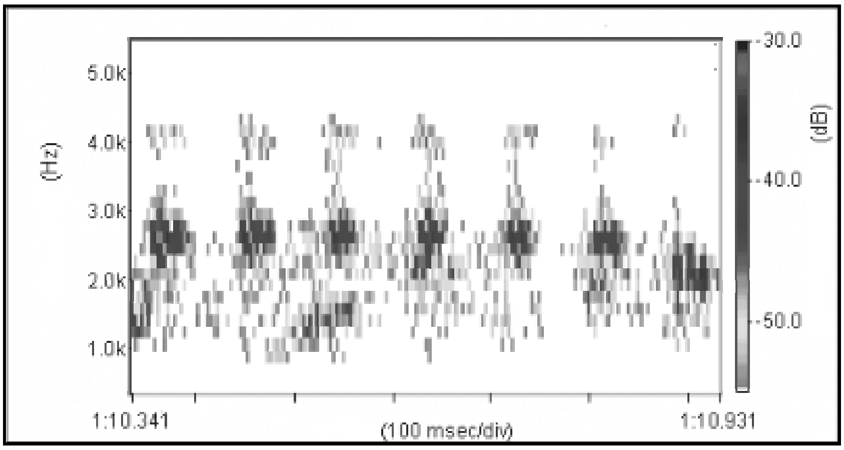

Figure 4. *Rana ridibunda* mating call spectrogram

The medium amplitude spectrums (Figure 5.) computed for each species summarise the data of 30 *Rana lessonae* mating calls, 22 *Rana esculenta* mating calls and 33 *Rana ridibunda* mating calls.

The maximum average amplitude for all the species is approximately between 500 – 3000 Hz. The medium spectrum of *Rana lessonae* has the highest amplitude at 2067.18 Hz. The next amplitude, below the highest one, corresponds to 689.06 Hz and appears as a clearly differentiated smaller peak. *Rana ridibunda* mating call maximum average amplitude appears as a peak corresponding to the frequency of 2067.18 Hz just like in *Rana lessonae*. The difference consists of the absence of the second smaller peak. *Rana esculenta* maximum average amplitude is distributed at the same frequency as in the other two species. The second amplitude peak of *Rana esculenta* mating call is present but at 1205.85 Hz, which is different than the second frequency peak of *Rana lessonae* mating call.

Again, this graphic approach reveals the hybrid status of *Rana esculenta*.

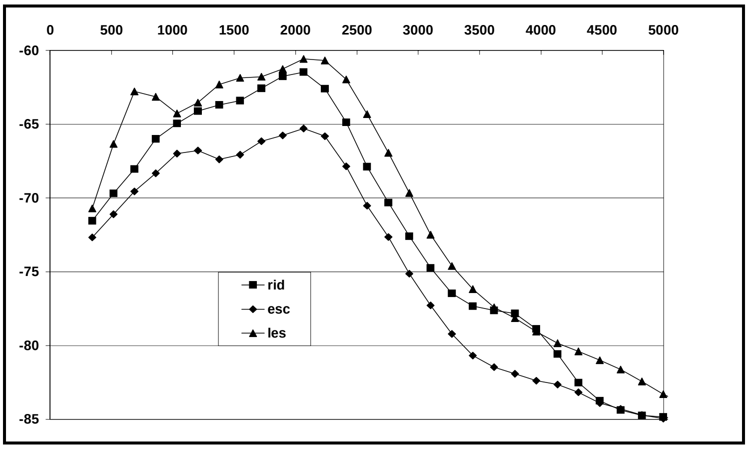

Figure 5. Mating calls medium spectrums of the *Rana esculenta* complex (les – *Rana lessonae*, esc – *Rana esculenta*, rid – *Rana ridibunda*)

Discriminant analysis results show weather duration and amplitude of the frequencies (up to 3000Hz) are useful for species discrimination and how distant are the studied species if mating calls are taken into account.

The first step was the discriminant analysis of *Rana lessonae, Rana esculenta* and *Rana ridibunda* mating call variables. Thus, the most powerful discriminant variable was call duration followed by the relative amplitudes that correspond to the following frequencies: 0.68 kHz, 0.51 kHz, 0.86 kHz (Figure 6.).

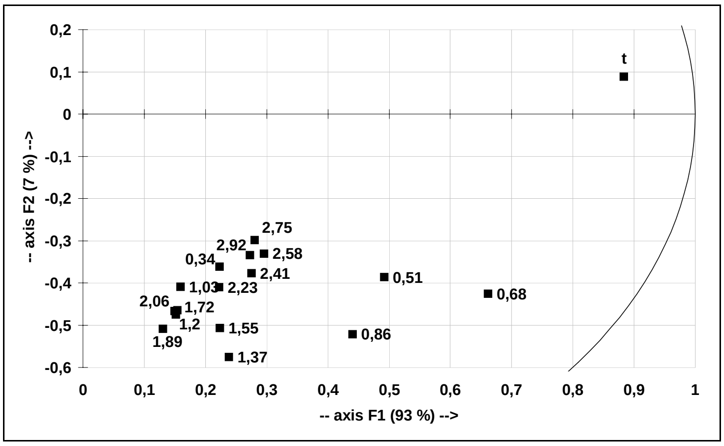

Figure 6. Discriminant potentials of the average amplitudes and duration of *Rana esculenta* complex (t – call duration)

The distances among species (Figure 7.) are significantly different (Table 1). Consequently, call duration and the amplitudes corresponding to the above mentioned frequencies can distinguish the species. The classification based upon call analysis has an error rate of 8.24% (Table 3.).

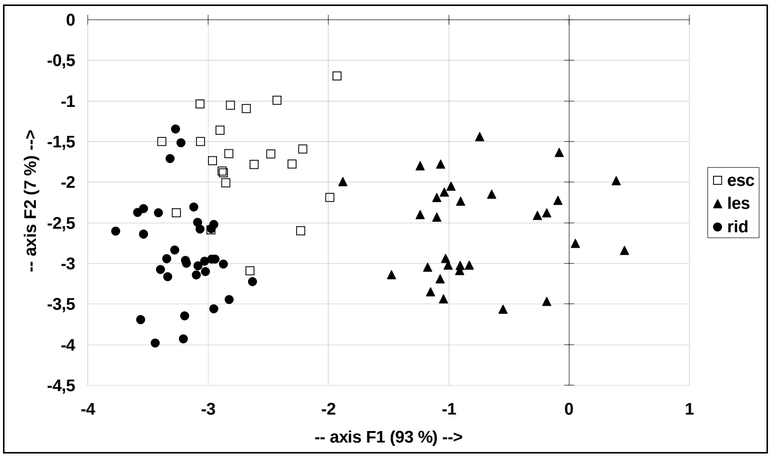

Figure 7. Distance between the average amplitudes and duration components of *Rana esculenta* complex

Table 2. Discriminant analysis – distances and significance – of *Rana lessonae* (les), *R. esculenta* (esc), *R. ridibunda* (rid) mating call variables

Squares of the Mahalanobis distances between groups:			
	esc	les	rid
esc	0	23.956	4.884
les	23.956	0	34.916
rid	4.884	34.916	0
Fisher's F associated to the squares of the Mahalanobis' distances between groups:			
	esc	les	rid
esc	0	**12.162**	**2.648**
les	**12.162**	0	**23.810**
rid	**2.648**	**23.810**	0
In bold, significant values at the level of significance alpha=0.050 (one-tailed test)			

Table 3. Summary of reclassification resulted from discriminant analysis of *Rana lessonae* (les), *R. esculenta* (esc), *R. ridibunda* (rid) mating call variables

	to esc	to les	to rid	Sum
from esc	19 22.35%	0 0.00%	3 3.53%	22 25.88%
from les	1 1.18%	29 34.12%	0 0.00%	30 35.29%
from rid	3 3.53%	0 0.00%	30 35.29%	33 38.82%
Sum	23	29	33	85
	27.06%	34.12%	38.82%	100.00%
Apparent error rate 8.24 %				

The next two steps of the discriminant analysis aimed to outline which are the most discriminant mating call variables for the following pairs of species: *Rana lessonae* / *Rana esculenta* and *Rana esculenta / Rana ridibunda*. These pairs group the species that the most difficult to distinguish.

The discriminant analysis of *Rana lessonae* and *Rana esculenta* mating calls variables revealed that call duration and the amplitudes that correspond to 0.68 kHz, 0.86 kHz, 0.51 kHz, 1.37 kHz and 1.55 kHz are the most powerful in species differentiation (Figure 8.). Distance analysis (Figure 9.) - Malahanobis's square distance 21.844 – outlined a significant difference between the two species mating calls variables - Fisher's F for distances 11.09, probability to accept the assumption of non-significant distance between groups (Fisher's test) was less than 0.05. The reclassification resulted from discriminant analysis was similar to the initial one (Table 4.).

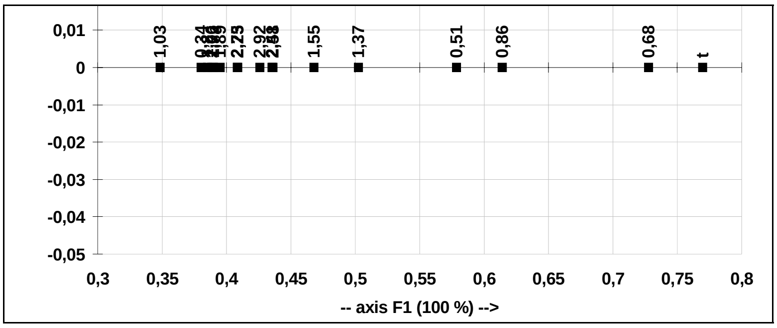

Figure 8. Discriminant potentials of the average amplitudes and duration of *Rana lessonae* and *Rana ridibunda* (t – call duration)

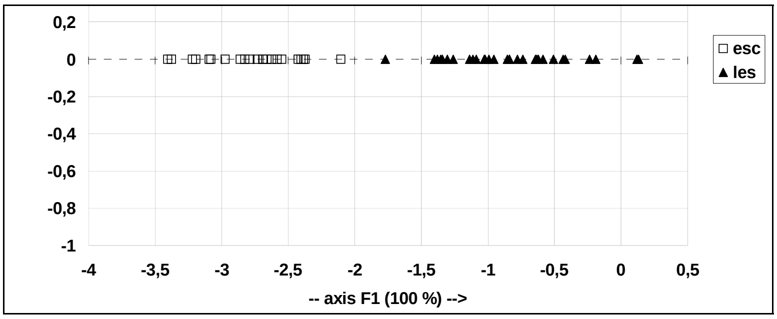

Figure 9 Distance between the average amplitudes and duration components of *Rana lessonae* and *Rana esculenta*

Table 4. Summary of reclassification resulted from discriminant analysis of *Rana lessonae* (les), *R. esculenta* (esc) mating call variables

	to esc	to les	Sum
from esc	22 42.31%	0 0.00%	22 42.31%
from les	0 0.00%	30 57.69%	30 57.69%
Sum	22 42.31%	30 57.69%	52 100.00%
Apparent error rate: 0.00 %			

The discriminant analysis of *Rana esculenta* and *Rana ridibunda* mating calls duration and the amplitudes, which corresponding to 1.37 kHz, 0.86 kHz, 1.89 kHz, are the most discriminant variables (Figure 10). Distance analysis (Figure 11) - Malahanobis's square distance 10.43 – outlined a significant difference between the two species mating calls relative amplitudes - Fisher's F for distances 5.65, probability to accept the assumption of non-significant distance between groups (Fisher's test) was less than 0.05. The reclassification resulted from discriminant analysis has an error probability of 3.64% (Table 5).

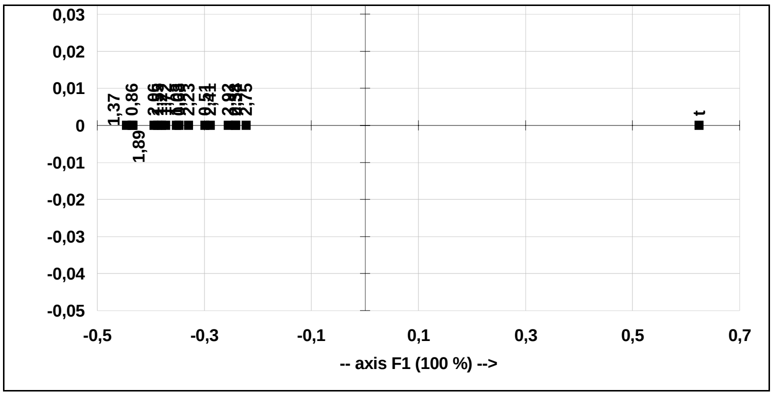

Figure 10. Discriminant potentials of the average amplitudes and duration of *Rana esculenta* and *Rana ridibunda* (t – call duration)

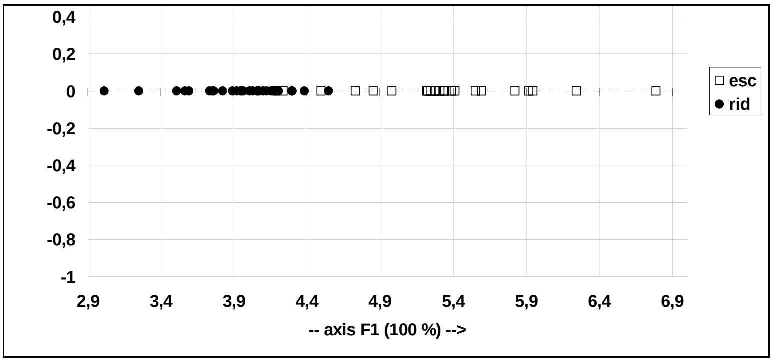

Figure 11. Distance between the average amplitudes and duration components of *Rana esculenta* and *Rana ridibunda*

Table 5. Summary of reclassification resulted from discriminant analysis of *R. esculenta* (esc), *R. ridibunda* (rid) mating call amplitudes

	to esc	to rid	Sum
from esc	20 36.36%	2 3.64%	22 40.00%
from rid	0 0.00%	33 60.00%	33 60.00%
Sum	20 36.36%	35 63.64%	55 100.00%
Apparent error rate: 3.64 %			

To conclude, the discriminant analysis proved that mating call duration and the amplitudes of certain frequencies of the mating calls could be used to identify the water frog complex species.

Conclusions

The mating call of *Rana lessonae* is longer than the mating call of *Rana esculenta*, which, in return, is longer than the mating call of *Rana ridibunda*.

Spectrograms show that the temporal structure of the mating call varies from approximately uniform in *Rana lessonae*, through jerky in *Rana esculenta*, to very jerky in *Rana ridibunda*.

The dominant frequencies of the mating calls of all species are between 500 – 3000 Hz, with 2 peaks in *Rana lessonae*, 1 peak in *Rana ridibunda* and two smoother peaks in *Rana esculenta*.

The medium relative amplitude spectrums confirm the above-mentioned differences.

All the characteristics of *Rana esculenta* mating calls are intermediate between those of *Rana lessonae* and *Rana ridibunda*.

The discriminant analysis revealed that the mating call duration is the most powerful discriminant characteristic. In addition, certain frequencies' amplitudes proved to be effective in water frog species differentiation (0.68 kHz, 0.86 kHz, 0.51 kHz, 1.37kHz, 1.89 kHz).

In conclusion, the bioacoustic analysis of the mating calls could be an alternative field method for identifying *Rana esculenta* complex species, especially *Rana lessonae* whose spatial distribution is little known in Romania.

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