

# Effects of Environmental Factors on Mating Call Characteristics of the Marsh Frog *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Ranidae) in Bulgaria

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**Abstract:** We studied mating calls of the marsh frog, *Pelophylax ridibundus*, from 12 geographically distinct populations from across Bulgaria. Recordings were made during the mating season (April-June) of 2011-2012. We tested whether the mating call was affected by environmental parameters like altitude, water and air temperature, climate zone, water body type and coexistence with closely related species. For the comparative analysis we used uni- and multivariate statistical methods and the following characteristics: dominant frequency, frequency modulation and tuning, call energy and relative amplitude of the harmonics. ANOSIM, NPMANOVA, rank-ordinal correlation and Spearman rank correlation techniques were performed in order to test for differences between and within localities. The results demonstrated that there was a significant correlation between the assigned environmental parameters and most of the studied call characteristics. The greatest number of correlations was registered in the parameters related to call energy and dominant frequency. Overall, populations exhibited great similarity, but also some differences which could be due to complex influence of the various environmental parameters. These findings are discussed in relation with habitat characteristics, coexistence and early stages of dialect development.

**Keywords:** Environment, mating call, *Pelophylax ridibundus*, inter-population variance

## Introduction

Acoustic communication plays an important role in anuran reproductive behaviour. During the breeding season males of most anuran species produce species-specific advertisement calls that coordinate the social behaviour of both males and females (RAND 1988, WELLS 1977). In consequence, the study of vocalisation is extensively used to elucidate taxonomic and phylogenetic problems. It is also considered an important characteristic for species identification (WELLS 2007).

Studies on bird and mammal acoustic communication have shown that their songs and calls are often structurally adapted to local habitat conditions (BROWN, GOMEZ 1992). Variations in the advertise-

ment call of anuran species may be due to factors, such as air and/or water temperature, size of the calling male, physiological condition, and social context, in which the call is emitted (BOSCH, DE LA RIVA 2004). For instance, physical characteristics of the environment may influence long-range acoustic signals, favouring those features that enhance their detection by the receiver (BOSCH, DE LA RIVA 2004). However, neither comparative (ZIMMERMAN 1983) nor experimental studies (KIME *et al.* 2000) have established a certain influence of environmental parameters over call characteristics. Nevertheless, there have been studies that attributed difference in temporal patterns to environmental parameters (RYAN, SULLIVAN

1989). According to BOSCH, DE LA RIVA (2004) most spectral and temporal patterns of the calls are determined either by morphology, physiology or behaviour. Frequency-related parameters of the call in some South American dendrobatid frogs are affected by the body size (PRÖHL *et al.* 2007, KAEFER *et al.* 2012). Thus, the physical characteristics of the environment may have a more subtle influence on call characteristics, especially on comparing populations from ecologically different or geographically distant habitats.

Environmental conditions may affect anuran call characteristics and it is expected that the acoustic properties vary with temperature and other climatic variables because of the animals' ectothermic condition. Several studies have demonstrated that some environmental parameters affect the calls. High temperature, for example, is well known to be directly related to the increase in the number of pulses and pulse groups, as well as to a decrease in overall call duration. Temperature may even affect the frequency preference of the receiver, although it does not seem to alter significantly the frequency of the emitted calls (GERHARDT, MUDRY 1980).

The marsh frog, *Pelophylax ridibundus* (Pallas, 1771), is distributed mainly in central and eastern Europe and the Balkans, while the taxonomic status of the Asian populations is unclear (PLÖTNER 2005, LYMBERAKIS *et al.* 2007, AKIN *et al.* 2010). Due to its wide range this species exhibits considerable variability in its characteristic traits. The southern margin of distribution is insufficiently known because many new species are described from different geographic populations in these parts of its range (NEVO, SCHNEIDER 1983, SCHNEIDER *et al.* 1993, SCHNEIDER, SINSCH 1999). Previous studies have focused on various call characteristics, but mainly for taxonomic reasons (SINSCH, SCHNEIDER 1996, SCHNEIDER, SINSCH 1999). Data on the environmental influence have been scarce, even more so considering the wide range of the species and the perspective of early stages development of dialects among populations. While it is known that geographic distance between populations has an influence on call characteristics (WELLS 2007), additional research is needed regarding the influence of the aforementioned local environmental parameters. In our study we tested the hypothesis whether the mating call was affected by some environmental parameters as climate, water body type, altitude, water and air temperature (near ground level). A study by HOBEL, GERHARDT (2003) suggests that interactions between different species can also play a role in the evolution of communication and call characteristics. Therefore,

we also included coexistence with congeners: *P. lessonae*, *P. esculentus*, *P. cf. bedriagae*, and *P. kurtmuelleri*, as an additional factor. According to HOTZ *et al.* (2013), *P. cf. bedriagae* reaches to the west at least as far as the Nestos River region. Some personal observations (Tzankov, Popgeorgiev, in prep.) have confirmed its presence in the southeastern part of Bulgaria (the Eastern Rhodope Mountains and Southern Black Sea coast). Although the taxonomic status of *P. kurtmuelleri* is unclear (LYMBERAKIS *et al.* 2007, PLÖTNER *et al.* 2008), recently this taxon was treated as a full species based on the nuclear sequences (PLÖTNER *et al.* 2012), cytogenetic differences and centromeric hybridisation pattern (MARRACCI *et al.* 2011). Therefore, in the present study this taxon is assumed to be a separate species as well.

## Material and Methods

We recorded and analysed 627 mating calls of the marsh frog (*P. ridibundus*) from 12 geographically distinct populations from across Bulgaria (Table 1). The signals that were emitted by two or more animals simultaneously or distorted by noise were not analysed. All individuals were adult with similar body size of 60-80 mm. In our previous study in South-West Bulgaria (LUKANOV *et al.* 2013) *P. kurtmuelleri* and *P. ridibundus* were distinguished based on multiple call characteristics, such as the number, duration, interval, and period of the pulse group, the energy and frequency of the call, etc.

All samples were taken during the mating season (April-June 2011). In the village of Rupite the presence of warm mineral springs allows for all-year activity, so that we were also able to make some recordings during the early spring (March 2011) and mid-winter (December 2011).

Air (near ground level) and water temperatures of the localities were measured because they have great influence on amphibian activity. The temperatures were measured using a digital water-resistant thermometer with an accuracy of 0.1 of the degree. Call recordings and measurements were taken once per day during the midday hours (14-16 h). The vocal activity was recorded using an Olympus LS-5 linear PCM recorder and an Olympus ME-31 shotgun microphone. We recorded the mating calls in a WAV-PCM mode with sampling frequency of 44.1 kHz, 20 - 21.000 Hz and 24 bit resolution. The recordings were processed with the computer program Soundruler V. 0.9.6.0. (GRIDI-PAPP *et al.* 2007).

For the statistical analyses we used the following sound characteristics (Table 2): the energy between

**Table 1.** Coordinates of studied localities and number of calls recorded. Pk – *Pelophylax kurtmuelleri*, Pb – *P. bedriagae*, Pe – *P. esculentus*, Pl – *P. lessonae*

Locality	Abbreviation	North	East	Altitude, m a.s.l.	Calls	Coexistence
Kresna	kr	41°43'15"	23°09'36"	172	73	Pk
Rupite	ru	41°27'31"	23°15'48"	88	142	Pk
Kolets	k	41°51'23"	25°20'21"	307	30	Pb
Aleksandrovo	a	42°19'10"	26°52'11"	164	25	-
Strandza	s	42°04'31"	27°00'20"	450	18	-
Plovdiv	pl	41°11'36"	25°45'49"	164	15	-
Ribaritsa	r	42°51'00"	24°21'24"	562	58	-
Popovski Heights	po	43°21'28"	26°00'22"	324	39	-
Oriahovo	o	43°43'18"	24°01'07"	28	68	Pe/Pl
Nova Cherna	n	44°01'33"	26°31'04"	12	14	Pe/Pl
Pancharevo	p	42°36'19"	23°24'14"	589	185	-
Pastrina	pa	43°25'28"	23°20'48"	272	27	-

**Table 2.** Description of the studied variables with abbreviations

Variable name	Description
Ener_Peak-90_End	Energy between final peak:90% amplitude
PulseDomFreq	Dominant frequency of the pulse
PulseHalfFM	Prop of duration to reach half frequency modulation
Tuning-6dBSPL	Tuning: peak freq/bandwidth at 50% peak ampl (Q-6dBSPL)
Tuning-20dBSPL	Tuning: peak freq/bandwidth at 10% peak ampl (Q-20dBSPL)
relAmpl_H1	Relative amplitude of harmonic 1
relAmpl_H2	Relative amplitude of harmonic 2
relAmpl_H3	Relative amplitude of harmonic 3
relAmpl_H4	Relative amplitude of harmonic 4
relAmpl_H5	Relative amplitude of harmonic 5

final peak: 90% amplitude (Ener\_Peak-90\_End), dominant frequency of the pulse (PulseDomFreq), prop of duration to reach half frequency modulation (PulseHalfFM), tuning: peak frequency/ bandwidth at 50% peak amplitude (Tuning-6dBSPL), tuning: peak frequency/ bandwidth at 10% peak amplitude (Tuning-20dBSPL), relative amplitude of harmonic 1 (relAmpl\_H1), relative amplitude of harmonic 3 (relAmpl\_H3), relative amplitude of harmonic 4 (relAmpl\_H4), and relative amplitude of harmonic 5 (relAmpl\_H5).

We tested whether the above call characteristics were affected by the following environmental parameters: geographic latitude (X) and longitude (Y), altitude (A), water (WT) and air (AT) temperature, climate zone (CZ), water body type (WBT), as well as coexistence with closely related species (C). The latter three variables were categorised as follows: (CZ): 1- moderate continental, 2- transitional continental and 3- continental Mediterranean (accord-

ing to KOPRALEV 2002); (WBT): 1- lotic, 2- lentic up to 10 m in diameter, 3- lentic between 10-50 m, 4- lentic larger than 50 m; (C): 1- *P. ridibundus*, 2- *P. kurtmuelleri*, 3- *P. bedriagae* 4- *P. esculentus*, 5- *P. esculentus* and *P. lessonae*.

Two non-parametric multivariate techniques were chosen in order to test more accurately the differences between and within localities. The first technique, ANOSIM (Analysis of Similarities), is a non-parametric test of significant difference between two or more groups, based on any distance measure (CLARKE 1993). Large positive *R* (up to 1) signifies dissimilarity between groups. The second technique, NPMANOVA (Non-Parametric MANOVA), is a non-parametric test of significant differences between two or more groups, based on any distance measure (ANDERSON 2001). For both tests the significance was computed by permutation of group membership, with 9999 replicates. Pairwise ANOSIMs and NPMANOVAs between all pairs of

**Table 3.** Pairwise comparison of call characteristics: p values based on uncorrected significance (p; in bold) and sequential Bonferroni correction (in bold and italic). Above diagonal – ANOSIM; below diagonal – NPMANOVA. For abbreviations of localities see Table 1; s.B. – sequential Bonferroni correction

	n	o	p	pa	pl	K	kr	a	pv	r	ru	s	p	s.B.
n	0	<b>0.0409</b>	0.3693	0.0853	<b>0.0101</b>	<b>0.019</b>	0.1947	<b>0.0102</b>	<b>0.0296</b>	<b>0.0087</b>	<b>0.0054</b>	0.4959	7	<b>0</b>
o	0.2579	0	<b>0.0041</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	0.5997	0.1126	<b>0.0001</b>	<b>0.0001</b>	0.3002	0.1084	7	<b>2</b>
p	0.0523	<b>0.0149</b>	0	<b>0.0013</b>	<b>0.0003</b>	<b>0.0001</b>	0.3918	<b>0.0003</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0001</b>	0.9116	<b>8</b>	<b>7</b>
pa	<b>0.0004</b>	<b>0.0219</b>	0.2155	0	<b>0.0205</b>	<b>0.0342</b>	<b>0.0001</b>	<b>0.0001</b>	0.2487	<b>0.0478</b>	<b>0.0001</b>	<b>0.0029</b>	<b>9</b>	<b>6</b>
pl	<b>0.001</b>	<b>0.0009</b>	<b>0.002</b>	<b>0.0393</b>	0	0.5414	<b>0.0001</b>	<b>0.0001</b>	0.317	0.6364	<b>0.0001</b>	<b>0.0004</b>	<b>8</b>	<b>6</b>
k	0.1269	<b>0.0001</b>	<b>0.0001</b>	<b>0.0025</b>	0.5863	0	<b>0.0017</b>	<b>0.0001</b>	0.1431	0.4775	<b>0.0001</b>	<b>0.0005</b>	<b>8</b>	<b>6</b>
kr	0.1644	0.8345	0.6647	<b>0.0012</b>	<b>0.0001</b>	<b>0.044</b>	0	0.1855	<b>0.0002</b>	<b>0.0012</b>	0.3062	0.3857	<b>5</b>	<b>5</b>
a	0.171	0.094	<b>0.0069</b>	<b>0.0001</b>	<b>0.0003</b>	<b>0.0001</b>	0.7109	0	<b>0.0001</b>	<b>0.0001</b>	0.2396	<b>0.017</b>	<b>8</b>	<b>6</b>
pv	<b>0.0229</b>	<b>0.0001</b>	<b>0.0014</b>	0.6773	0.9455	<b>0.0165</b>	<b>0.009</b>	<b>0.0001</b>	0	0.2595	<b>0.0001</b>	<b>0.0008</b>	<b>7</b>	<b>6</b>
r	<b>0.0036</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0195</b>	0.9195	<b>0.0317</b>	<b>0.0001</b>	<b>0.0001</b>	<b>0.0369</b>	0	<b>0.0001</b>	<b>0.0005</b>	<b>8</b>	<b>6</b>
ru	0.0759	0.2325	<b>0.0001</b>	<b>0.0004</b>	<b>0.0001</b>	<b>0.0001</b>	0.8033	<b>0.0268</b>	<b>0.0001</b>	<b>0.0001</b>	0	<b>0.015</b>	<b>8</b>	<b>6</b>
s	0.3085	0.7374	0.3556	<b>0.0003</b>	<b>0.0003</b>	<b>0.0064</b>	0.5352	0.32	<b>0.0023</b>	<b>0.0001</b>	0.4232	0	<b>7</b>	<b>4</b>
<b>p</b>	<b>4</b>	<b>6</b>	<b>7</b>	<b>9</b>	<b>8</b>	<b>9</b>	<b>5</b>	<b>7</b>	<b>9</b>	<b>10</b>	<b>7</b>	<b>5</b>		
<b>s.B.</b>	<b>2</b>	<b>4</b>	<b>2</b>	<b>4</b>	<b>6</b>	<b>3</b>	<b>2</b>	<b>5</b>	<b>3</b>	<b>6</b>	<b>6</b>	<b>3</b>		

**Table 4.** Rank/ordinal correlation between call variables and environmental parameters; p values based on Monte Carlo permutation test. Correlation coefficients are in parenthesis. Marked correlations are significant at p < 0.05. For abbreviations, see Material and Methods

Variable	CZ	C	WBT
Ener_Peak90_End	0.0001 (0.2505)	0.0061 (0.1077)	0.0003 (-0.1454)
PulseDomFreq	0.0001 (0.2692)	0.0001 (0.2350)	0.0001 (-0.4750)
PulseHalfFM	0.1668 (-0.0559)	<b>0.0482 (-0.0785)</b>	<b>0.0427 (0.0808)</b>
Tuning6dBSPL	0.3339 (0.0386)	0.7236 (0.0139)	<b>0.0057 (-0.1119)</b>
Tuning20dBSPL	<b>0.7212 (0.0142)</b>	0.0832 (-0.0687)	<b>0.0494 (-0.0791)</b>
relAmpl_H1	0.002 (0.1215)	<b>0.0456 (0.0798)</b>	<b>0.0001 (-0.1812)</b>
relAmpl_H3	0.2801 (0.0437)	0.1761 (-0.0542)	<b>0.0151 (0.0972)</b>
relAmpl_H4	<b>0.0001 (0.1713)</b>	<b>0.0001 (0.2338)</b>	0.3823 (0.0347)
relAmpl_H5	0.0001 (0.2434)	0.0001 (0.3281)	0.0001 (-0.3322)

**Table 5.** Spearman rank correlations coefficients between call variables and environmental parameters. Marked correlations are significant at p < 0.05. For abbreviations, see Material and Methods

Variable	X	Y	A	WT	AT
Ener_Peak90_End	<b>-0.206</b>	<b>-0.280</b>	<b>-0.212</b>	0.001	<b>-0.110</b>
PulseDomFreq	<b>-0.251</b>	<b>-0.315</b>	<b>-0.191</b>	<b>0.118</b>	<b>-0.121</b>
PulseHalfFM	<b>0.084</b>	<b>0.229</b>	<b>0.081</b>	-0.040	<b>0.114</b>
Tuning6dBSPL	-0.020	<b>-0.125</b>	-0.032	-0.036	-0.076
Tuning20dBSPL	<b>-0.102</b>	<b>-0.164</b>	<b>0.118</b>	-0.016	<b>-0.145</b>
relAmpl_H1	<b>-0.195</b>	<b>-0.137</b>	0.064	0.041	<b>-0.090</b>
relAmpl_H3	<b>-0.144</b>	-0.022	<b>0.116</b>	<b>0.111</b>	-0.010
relAmpl_H4	<b>-0.098</b>	-0.017	<b>-0.227</b>	0.074	0.068
relAmpl_H5	<b>-0.232</b>	<b>-0.139</b>	<b>-0.215</b>	0.065	<b>-0.079</b>

groups were used as a post-hoc test (at  $p < 0.05$ ). The step-down sequential Bonferroni correction was used to multiply the  $p$  values with the number of comparisons. We also used rank-ordinal correlation analyses in order to determine whether environmental rank parameters (CZ, WBT, C) were related to call characteristics. To correct for the occurrence of false positives, a Monte Carlo permutation test with 9999 random replicates was performed. For studying the relationships between call characters and the continuous environmental parameters (X, Y, A, WT and AT), Spearman rank correlation ( $\rho$ ) was chosen because of the uneven distribution pattern of the sampled data. A discriminant function analysis was performed in order to determine the Mahalanobis distances between localities. All tests were performed using the computer programs Statistica V. 7.0 (STATSOFT, INC. 2004) and Past 2.17 (HAMMER *et al.* 2001).

## Results

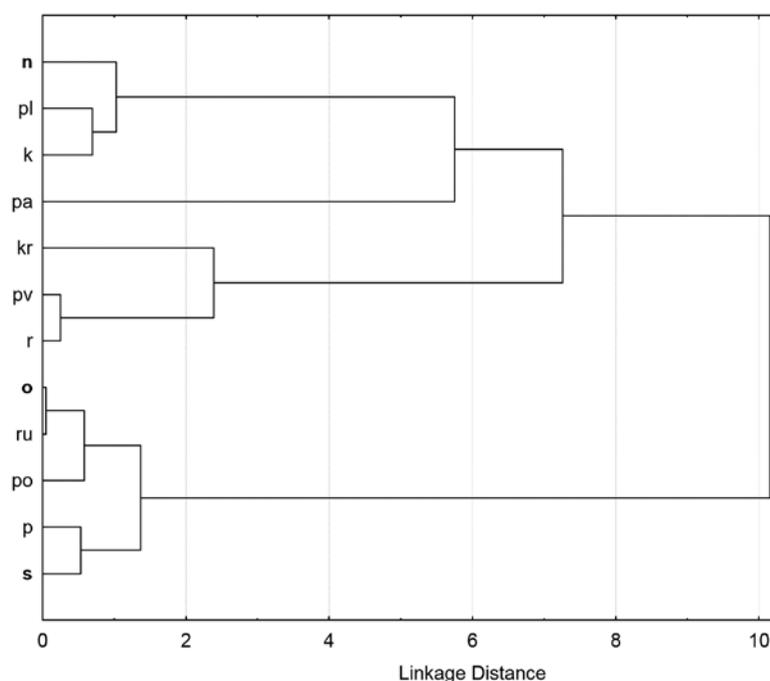
The combined results from the ANOSIM and NPMANOVA tests demonstrated that some call characteristics differed among the designated groups. Localities Nova Cherna and Strandza had the least differences when compared to the other localities. The least variable call characteristics were the pulse half frequency modulation (PulseHalfFM) and peak frequency/ bandwidth at 10% peak amplitude

(Tuning-20dB SPL). The other call characteristics varied more or less uniformly across the studied localities. There was no indication that the geographic position was a factor in the detected differences (for details see Table 3). The localities with the highest number of differences in variables were those characterised by lentic ponds (Ribaritsa, Pastrina). At the same time, the localities with lotic habitats, such as Nova Cherna and Strandza, showed a lower number of differences in variables.

According to the rank-ordinal correlation analysis all frequency-related call characteristics displayed statistically significant correlation with the three rank environmental parameters (Table 4). Similarly, a significant correlation was also present in some harmonics and energy characteristics.

The Spearman rank correlation test revealed statistically significant correlations between the continuous environmental parameters and most of the studied call characteristics (Table 5). Parameter X demonstrated strong correlation with eight of the nine variables; parameters Y and A correlated with seven, parameter WT – with six, while parameter AT was the least important with only two statistically significant correlations established. The dominant frequency was the only variable that displayed statistically significant correlation with both continuous and rank environmental parameters.

The results from the discriminant analysis did not reveal certain combination of features that could



**Fig. 1.** A tree diagram (Ward's method) presenting the Mahalanobis distance between populations. For abbreviations, see Table 1

clearly distinguish between the designated groups. However, some differences between groups could be still established - the greatest squared Mahalanobis distance was 6.6 between localities Rupite and Pastrina. In the classification matrix of predicted vs. observed classification the highest percent correctly assigned calls was 71.2% for locality Pancharevo. A cluster analysis based on the Mahalanobis distances did not provide definitive results (Fig. 1). The distant localities, such as Rupite and Oriahovo, were assigned as sister groups, the greatest linkage distance being between localities Nova Cherna and Strandza.

## Discussion

The results obtained indicate that the studied environmental parameters have weak but observable effect on at least some of the analysed call characteristics. The examined populations did not display great variations, suggesting a low level of differences between them. This similarity was not unexpected considering the small territory of Bulgaria. We could propose several explanations of the differences that were significant. The type of the water body had the highest number of significant correlations of the rank environmental parameters, which implies that it had the greatest influence on the call variability. A possible reason could be the differences in habitat conditions - lentic/ lotic ecosystems, pond size, vegetation, etc. In the cluster analysis some populations from slow flowing lotic habitats were found to be related; however, because of various discrepancies we cannot draw any definitive conclusions from that finding. The fact that, in general, the lentic habitats displayed more differences than the lotic habitats should be taken into account. Factors as vegetation, temperature, water current (or lack of it), etc. influence the call characteristics. BOSCH, DE LA RIVA (2004) reported that the physical complexity of the microhabitat can provide an explanation of a small portion of the call variability in several South American species. They found, similarly to our results, the dominant frequency modulation to be related to environmental parameters. The frog populations that inhabit different environments can diverge in their tolerance to environmental stressors and some evidence have accumulated that different amphibian populations are differently sensitive to the same environmental stressors (e.g. BRIDGES, SEMLITSCH 2000, PAHKALA *et al.* 2001). Since water and air temperatures were shown to have the least influence on the inter-population variability, we can conclude that the studied marsh frog populations do

not differ in their tolerance to these environmental parameters.

Coexistence with closely related species seemed to have weaker influence on call variation than we expected. The results of our previous study (LUKANOV *et al.* 2013) also showed clear differences between the mating call of *P. ridibundus* in allopatry compared to sympatry with *P. kurtmuelleri*, which we interpreted as a selection against mismatching. Considering that along the Danube River *P. ridibundus* lives in sympatry with the closely related *P. lessonae* and *P. esculentus*, we expected more pronounced differences from these populations. The small differences in vocalisation could be affected by two basic factors, the first being connected to the fact that *P. esculentus* is a product from hybridogenesis between *P. ridibundus* and *P. lessonae*, which means that these species interbreed on a great scale within their respective ranges. Therefore, as LODE, PAGANO (2000) pointed out, the hybridogenetic process may favour such hybrid-species matings that are required to maintain the hybrid. This would counter the character displacement mechanism that acts towards better differentiation between species. The second factor concerns the mating calls of both *P. lessonae* and *P. esculentus*. They are quite different from that of *P. ridibundus* in their temporal and spectral structure. Therefore, there is no immediate need for character reinforcement. LODE, PAGANO (2000) also did not find evidence for character reinforcement in several species of water frogs from populations from southern and western France. They supposed that the mating call in water frogs is rather an attractive sexual signal than an isolation mechanism.

ABT, REYER (1993) reported that *P. esculentus* females are easily attracted by calls produced by *P. lessonae* males, which supports the mentioned idea. Sympatry with other *Pelophylax* species may also have influence on the number of differences in variables among populations (SCHNEIDER *et al.* 1993).

Some authors revealed that the Danube River acts as a natural corridor for water frog dispersal instead of a natural barrier that isolates them (MIKULÍČEK, PISUT 2012). According to them, the population structure of *P. ridibundus* also shows higher genetic connectivity within water paths than between them, which suggests a limited overland dispersal and reflects the historical landscape structure associated with the distribution of the lost river branches. It is difficult to say whether this connectivity via water paths is relevant to our study. The tests showed no differences in variables between localities Nova Cherna and Oriahovo that are both situated near the Danube River and have similar character-

istics. At the same time the cluster analysis showed great differences between these sites and links them to dissimilar populations from the southwestern part of the country, Kresna and Rupite, respectively. In fact, in all those localities *P. ridibundus* coexist with other congeneric species, e.g. *P. lessonae* and *P. esculentus* at the Danube River localities and *P. kurtmuelleri* at southwestern localities.

The data can also be viewed in the context of a possible dialect development among populations. The strong correlation of the dominant frequency with all assigned environmental parameters seems to be in support of such a view. Previously, existence of dialects was suggested in the species *Pelodytes punctatus* from southern Portugal (PAILLETTE *et al.* 1992). That hypothesis can be rejected now, as later studies revealed a new species together with still undescribed species existing in this region (SÁNCHEZ-HERRÁIZ *et al.* 2000) with a well expressed genetic differentiation (VAN DE VLIET *et al.* 2012). In anurans, in contrast to birds, it is considered that there is no dialect imprinting or learning in calls (ASQUITH *et al.*

1988). Intraspecific variation in anuran call characteristics may reflect population genetic differentiation or simply the influence of the individual morphological variation (e.g. size), environmental factors (e.g. temperature), or local social interactions (PAILLETTE *et al.* 1992). A study by WILCZYNSKI *et al.* (1992) of the dominant frequency in *Acris crepitans* found inter-population changes in calls and basilar papilla tuning in support of the idea of local inter-population differentiation. The hypothesis of those authors explained the coincident changes in female mate choice that would be directed toward males from the female home population with low frequency calls or toward males from other populations with average calls lower in frequency than those in female home population.

In our present study the populations exhibited great similarity, but also some differences which could be due to complex influence of various environmental parameters. Understanding the causes and consequences of these variations poses a challenge for further research.

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