

THE MATING CALL OF *PELODYTES IBERICUS* (ANURA, PELODYTIDAE)

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Acoustic communication is an important feature of anuran social behaviour. In fact, the species-specific mating call is the major pre-mating reproductive isolating mechanism in anurans (Blair, 1958; Gerhardt, 1974; Asquith *et al.*, 1988). Females are attracted almost exclusively to the call of a conspecific male, thus reducing the chances of hybridization. This acoustic specificity provides a tool that has been used extensively to elucidate taxonomic problems. Cryptic species may be identified through the analysis of their mating calls (Crespo *et al.*, 1989; Márquez & Bosch, 1995, 1996).

The Pelodytidae are an ancient family of frogs, originally classified as one genus with two distinct species: *Pelodytes caucasicus* Boulenger (1896) and *P. punctatus* Daudin (1802). The former species is known to occupy the Caucasus region, while the second is distributed over a large area, ranging from the Iberian Peninsula to France, with small extensions to Belgium, Luxembourg and Italy (Van den Elzen, 1975, 1976). Recently, a new species of the family, *P. ibericus*, was described, being endemic to the Iberian Peninsula (Sánchez-Herraiz *et al.*, 2000).

Few studies have analysed the mating call of the Pelodytidae. To our knowledge, the only data referring to the vocalization of *P. caucasicus* are those of Steiner (1968), who described the vocalization of this species as being composed of 2 or 3 multipulsed notes. The acoustic signals of *P. punctatus* have been studied by Hotz (1971) in Liguria (Italy) and by Van den Elzen (1975, 1976) in the Camargue (France). *Pelodytes punctatus* has an advertisement call consisting of two different multi-pulsed notes, named “A” and “B”, emitted in a specific order (Van den Elzen, 1975). In the Camargue

and Liguria, notes “A” and “B” are emitted in series of pairs “A-B”. Both notes can be divided into two separate parts: in the first part, when the pulses increase in intensity (rising phase), the pulse rate is slow. The pulse rate then undergoes a sudden acceleration in the second part of each note, when the pulses decrease in intensity (falling phase).

Paillette *et al.* (1992) analysed the mating calls of a population of *Pelodytes* from the Algarve (southern Portugal) and found a different syntax, as compared with those from the Camargue and Liguria. In the Portuguese population the succession of notes always begins with an “A”, but there is frequently more than one “B” in each sequence. The number of “B”s per sequence may be up to seven, which the latter authors attributed to a different geographical dialect.

Differences among Iberian *Pelodytes* species are evident at morphological, anatomical and genetic levels (Sánchez-Herraiz *et al.*, 2000). Before the description of *P. ibericus* as a separate species, some differences relating to mating calls were also found between *P. punctatus* from France and a population of *Pelodytes* from southern Portugal (Paillette *et al.*, 1992). As shown by Paillette *et al.* (1992), these differences include the number of repetitions of “B” notes in a single call sequence, as well as some temporal and spectral features.

In this paper we describe and quantify temporal and spectral characteristics of the advertisement call of males of *P. ibericus*. This bioacoustical information complements the analyses made by Paillette *et al.* (1992) and the description of the new species, which is based on morphological and genetic characters (Sánchez-Herraiz *et al.*, 2000).

Male mating calls were recorded in December 1995 from populations of *P. ibericus* in Mértola (UTM 29SPB16); Almada de Ouro (UTM 29SPB33), southeastern Portugal; and in Córdoba, Southern Spain (UTM 30SUH33). In Portugal, field recordings were made using a Uher 815 microphone connected to a Uher 4000 recorder, at a tape speed of 19 cm/s. In Spain, field recordings were made using an AKG D900 microphone connected to a Sony WM D6C recorder. Air and water temperatures from where the animals were calling were measured immediately after recording, as temperature is the main environmental factor affecting the calls (e.g. Schneider, 1974; Schneider & Nevo, 1972; Gerhardt, 1978; Gerhardt & Mudry, 1980; Paillette, 1986; Crespo, 1981). In the Spanish population, individuals were captured after being recorded and the mass of each (to the nearest 0.5 g) was determined with a Pesola spring balance, and their snout-vent-length (SVL) was determined to the nearest 1 mm by pressing the frog flat against a ruler.

Recordings were processed with Sound Tools hardware (Digidesign Inc.) in an Apple MacIntosh IIfx. Digitization was completed at a sampling frequency of 44.1 kHz and 16 bit resolution in the Spanish population, 22.05 kHz and 8-bit resolution in the Portuguese recordings, using Sound Designer II software (version

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2.5) and Sound Tools hardware (Digidesign Inc.). A band-pass filter (600-3500 Hz) was used to improve measurements. Signalyze software (version 3.12, Infosignal Inc.) was used to obtain numerical information from waveforms and audiospectrograms. Frequency information was obtained through fast Fourier transformation (FFT) with a width of 3.3 ms and a frequency resolution of 300 Hz.

We analysed a total of 727 calls (2366 notes) from 54 males: 394 calls (1221 notes) from Mértola (15 males) 117 calls (452 notes) from Almada de Ouro (4 males) and 216 calls (693 notes) from Córdoba (35 males).

In a previous, unpublished study that included our two Portuguese populations (Pargana, 1998), a separate analysis was done for each "B" note within a sequence, in order to establish the homogeneity of this note and eliminate the possibility of there being different note types among successive "B" notes. The study concluded that there were no significant differences among "B" notes within a sequence (ANOVA, $P > 0.05$ for all variables with the exception of D_{rising} : $P = 0.02$ and D_{falling} : $P = 0.039$).

The following call variables were measured, for each note "A" and "B":

Note duration (D);

Number of pulses (Pul);

Number of pulses in the rising phase of the note (NP_{rising});

Number of pulses in the falling phase of the note (NP_{falling});

Duration of the rising phase of the note (D_{rising});

Duration of the falling phase of the note (D_{falling});

Interpulse duration at the beginning of the note (IP_{init});

Interpulse duration at maximum amplitude (IP_{max});

Interpulse duration at the end of the note (IP_{end});

Dominant frequency at maximum amplitude (F_{max});

Dominant frequency at the end of the note (F_{end});

Duration of the interval between "A" and "B" notes within a sequence (A-B);

Duration of the interval between subsequent "B" notes within a sequence (B-B);

Duration of the interval between calls (B-A).

Within-individual and between-individual coefficients of variation were calculated for all the variables, in order to find out which are "static" and which are "dynamic" (*sensu* Gerhardt, 1991). Static parameters are presumably those that may better characterize the specific call, because they remain fairly constant among individuals from a single population or species, at least within a calling session.

The general structure of the mating call notes of *P. ibericus* was generally similar to that described for the congeneric species *P. punctatus*, being composed of two, different, multi-pulsed notes, which we refer to "A" and "B" to simplify comparisons with the call of *P. punctatus*. The calls were emitted in a specific temporal order, always beginning with an "A", generally followed by one or more "B" notes. The modal number of "B" notes in a sequence was two, emitted in 42% of the se-

quences from Mértola, 45% of the calls from Almada de Ouro and in 33% of the calls from Córdoba (Table 1). Sometimes there were calls with no "B" notes at all, although these were rare (2% in Mértola, 3% in Almada de Ouro and Córdoba; Table 1). The maximum number of "B" notes in a call was 10, recorded from a male from Córdoba.

A characteristic waveform and audiospectrogram of the call of *P. ibericus* is shown in Fig. 1. The two notes are multi-pulsed, with a change in pulse rate within them, an acceleration occurring immediately after the peak of maximum amplitude; and thus it can be considered that notes have two different parts: a first in which the sound level increases progressively until maximum amplitude is reached and pulses are emitted at relatively wide intervals (rising phase), and a second (falling phase) in which the sound level decreases and the pulse rate increases slightly (Fig. 1).

The numerical parameters (mean, standard deviation and coefficient of variation) for the variables studied are shown in Table 2. Multivariate analysis of variance (MANOVA) using 11 acoustic variables (Table 2) for the Córdoba population showed that both notes, A and B, differed significantly (Wilks' Lambda = 0.0834, $F_{11,58} = 57.938$, $P < 0.001$). A subsequent discriminant analysis provided 100% correct classification. Univariate analysis of variance (Table 3) revealed that most of variables differed.

Mean duration of note "A" was not significantly different to that of note "B", but note "A" had more pulses. In the "A" note, the duration of the rising phase was shorter, having more or less half the pulses of the falling phase. Although the number of pulses of the rising phase was also a half of the falling phase in the "B" note, the duration of both phases was very similar. Interpulse duration decreased throughout the note; however, within "B" notes it increased until the maximum amplitude peak, decreasing after that.

Concerning the spectral variables, frequency increased throughout both notes, and it was slightly higher in the "B" note. Within the "A" note it ranged from 1.4 to 2.5 kHz at the beginning and from 1.9 to 2.8 kHz at the end, and within the "B" note it ranged from 1.9 to 2.6

TABLE 1. Relative frequency of the number of "B" notes within a sequence (population average of individual relative frequencies).

Number of "B" notes per call	Almada de Ouro	Mértola	Córdoba
0	0.03	0.02	0.03
1	0.15	0.23	0.24
2	0.45	0.42	0.33
3	0.24	0.25	0.20
4	0.12	0.07	0.11
5	0.01	0.01	0.03
6	0.01	0	0.03
≥ 7	0	0	0.01

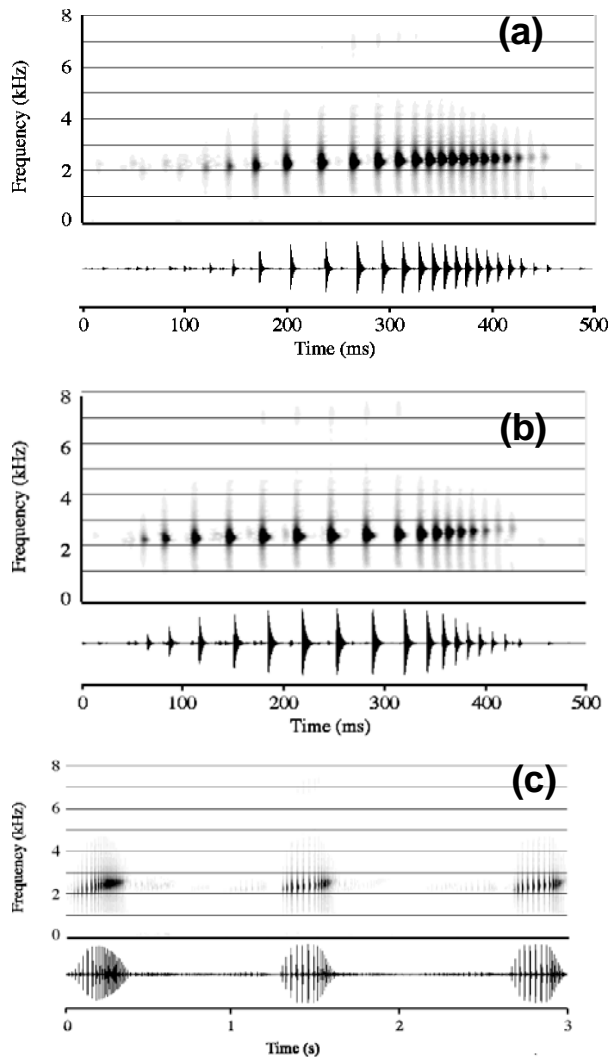


FIG. 1. Audiospectrogram (upper) and oscillogram (lower) of (a) a characteristic "A" note; (b) a characteristic "B" note; (c) a call (A-B-B). The male (SVL 39 mm) was recorded in Córdoba (water temperature 10 °C).

kHz at the beginning and from 2.0 to 2.9 kHz at the end. The increment (upwards frequency sweep between maximum amplitude and the end of the note) was higher in the "A" note than in "B" notes.

The interval between subsequent notes within a sequence was slightly shorter between an "A" and the first "B" than between subsequent "B" notes; it was about 10% of the interval between subsequent calls (Fig 1c).

The relationship between male size and sound parameters was studied in the population from Córdoba, where all recorded males were captured and measured. None of the correlations between male size (SVL) and call parameters was significant.

All the individuals from the samples from Portugal were recorded at similar temperatures (Mértola water temperature range: 14.5-15 °C, Almada de Ouro: all individuals recorded at 16 °C). Therefore, the effect of water temperature on call parameters was measured only in the sample from Córdoba, where the range of water temperatures was 8.4-17.0 °C. With the exception of the interval between calls (B-A), all of the temporal

variables (durations, interpulse, and inter-note intervals) and number of pulses were significantly correlated with water temperature. The regressions of mean note duration and number of pulses per note on water temperature are shown in Fig. 2. None of the frequency variables was significantly correlated with water temperature. The number of "B" notes per call was not significantly correlated with temperature.

The within-individual coefficients of variation of dominant frequency were the lowest of all parameters. Call duration and number of pulses per note exhibited a low coefficient of variation. The interval between note "A" and the first note "B" (A-B) also had a low value. The coefficient was higher between subsequent "B" notes and intervals between call sequences (B-A) were highly variable both between and within individuals (Table 2).

Unlike *P. punctatus*, whose mating calls are emitted in series of pairs "A-B" (Hotz, 1971; Van den Elzen, 1975, 1976), *P. ibericus* calls are composed of sequences with a variable number of "B" notes, most often having two "B" notes. None of the individuals recorded emitted only "A-B" calls. Van den Elzen (1976) provides only a rough estimate of the emphasized frequencies for *P. punctatus* for comparison (from 1.8 to 3.5 kHz divided into two bands; one at 1.8-2.1 kHz, and another at 2.8-3.5 kHz), which renders a quantitative comparison with our data impossible. The mean duration of the "A" note for *P. punctatus* from the Camargue (France) was 292.5 ms, and the mean duration of the "B" note was 276.0 ms at 17.5 °C, values that are within the population means from our data (Table 2) but higher than the expected durations for *P. ibericus* at that temperature (Fig. 2a).

Similarly to Paillette *et al.* (1992), who studied a population from Castro Marim (about 10 km away from Almada de Ouro), we found that the most frequent number of "B" notes per call was two, although the former authors found an even higher frequency of sequences with two "B"s (55%). In light of the study by Sánchez-Herraiz *et al.* (2000), and of our own results, we believe that what Paillette *et al.* (1992) considered to be a different dialect of *P. punctatus* from Southern Portugal was in fact the mating call of *P. ibericus*.

Márquez *et al.* (2001) did not find evidence of an increment of the number of "B" notes or call matching through male-male acoustical interactions, suggesting that social interactions are not the main cause of variability in the number of "B" notes.

The variability of the number of "B" notes in a sequence could also be related to a different function of the notes (Narins & Capranica, 1976; Capranica, 1977) or to an increment of informative content through redundancy (Duellman & Trueb, 1986).

Temperature, one of the most important environmental factors affecting call characteristics, may be discarded as a cause. Although temperature is positively correlated with metabolic rate and, consequently, with vocal activity, linear regression did not show any corre-

TABLE 2. Mean and coefficient of variation (CV) of call parameters of *P. Ibericus* for each population studied. “CV between” is a measure of between-individual variability and is calculated with the coefficients of variation of the mean values of the individual males; “CV within” is a measure of the within-individual within-calling period variation, calculated as the average of the individual coefficients of variation of each recording; *n* is the number of individuals. The identification of the acronyms of the call parameters are in the text. Units are ms for duration variables and Hz for frequency variables.

Note “A”		<i>D</i>	<i>NP</i>	<i>NP</i> _{raise}	<i>NP</i> _{fall}	<i>D</i> _{raise}	<i>D</i> _{fall}	<i>IP</i> _{init}	<i>IP</i> _{max}	<i>IP</i> _{end}	<i>F</i> _{max}	<i>F</i> _{end}	A-B	
Mértola (<i>n</i> =15)	Mean	192.6	22.7	7.5	15.1	83.3	109.4	12.7	10.9	6.1	2280.6	2424.8	489.3	
	CV _{between}	11.1	11.9	10.7	15.2	10.7	13.7	18.1	19.3	14.8	6.5	7.2	19.1	
	CV _{within}	4.4	5.6	10.9	8.2	11.5	8.2	11.1	15.2	7.6	2.4	2.4	13.3	
Almada D’Ouro (<i>n</i> =4)	Mean	151.4	21.4	7.5	13.9	67.5	83.9	12.2	7.9	5.3	2230.8	2364.1	426.0	
	CV _{between}	7.8	21.5	18.7	24.5	3.6	12.5	30.3	30.4	34.0	4.3	6.0	5.7	
	CV _{within}	4.8	5.2	13.2	9.0	13.0	8.8	11.1	15.1	5.4	2.1	2.7	8.1	
Córdoba (<i>n</i> =35)	mean	323.6	25.5	6.4	19.0	127.7	196.4	19.4	20.1	8.9	2031.7	2348.2	755.4	
	CV _{between}	23.4	14.9	15.6	16.8	27.0	24.7	18.6	21.4	21.3	3.8	4.5	19.9	
	CV _{within}	7.5	10.6	11.0	12.1	15.9	12.1	14.3	19.2	9.6	3.4	1.9	15.4	
Note “B”		<i>D</i>	<i>NP</i>	<i>NP</i> _{raise}	<i>NP</i> _{fall}	<i>D</i> _{raise}	<i>D</i> _{fall}	<i>IP</i> _{init}	<i>IP</i> _{max}	<i>IP</i> _{end}	<i>F</i> _{max}	<i>F</i> _{end}	B-B	B-A
Mértola (<i>n</i> =15)	Mean	193.5	16.3	5.5	10.8	96.4	97.2	16.3	28.3	7.8	2371.0	2481.2	546.5	7050.7
	CV _{between}	8.9	14.1	10.9	17.6	10.5	14.0	17.2	19.4	19.2	7.9	8.9	18.0	46.5
	CV _{within}	3.9	4.2	8.8	7.3	12.4	10.6	12.6	10.7	9.2	2.0	2.7	11.7	91.0
Almada D’Ouro (<i>n</i> =4)	Mean	156.7	13.0	5.5	8.4	88.7	67.9	18.2	22.5	6.5	2349.3	2441.9	473.9	3175.0
	CV _{between}	7.9	20.8	16.4	26.2	10.0	24.3	36.3	11.6	27.7	6.2	6.9	8.6	39.1
	CV _{within}	5.0	5.2	10.8	9.6	12.7	12.8	13.2	15.6	5.3	1.4	1.9	13.5	82.1
Córdoba (<i>n</i> =35)	Mean	317.5	17.3	7.5	9.7	211.8	105.9	22.2	36.6	10.4	2128.3	2334.0	862.7	2895.9
	CV _{between}	20.9	12.1	12.0	16.5	21.5	26.4	18.0	20.8	21.2	3.8	4.9	21.0	67.2
	CV _{within}	8.2	7.3	12.0	12.7	12.0	12.9	14.3	9.0	7.4	2.2	2.2	13.5	38.5

TABLE 3. Univariate analysis of variance for call variables comparing the notes A and B of *P. ibericus* from the Córdoba population. The estimates that are expressed in bold type remained significant after the sequential Bonferroni

	SS	MS	F	P
<i>D</i>	680.8	5062.29	0.13	0.715
<i>NP</i>	1159.3	9.27	125.00	<0.001
<i>NP</i> ^{raise}	21.4	0.9	23.78	<0.001
<i>NP</i> ^{fall}	889.8	93.85	9.48	<0.01
<i>D</i> ^{raise}	123777.9	1645.59	75.22	<0.001
<i>D</i> ^{fall}	143358.5	1612.02	88.93	<0.001
<i>IP</i> ^{init}	144.2	14.64	9.85	<0.01
<i>IP</i> ^{max}	4740.2	38.28	123.82	<0.001
<i>IP</i> ^{end}	34.0	4.06	8.37	<0.01
<i>F</i> ^{max}	163606.4	6385.56	25.62	<0.001
<i>F</i> ^{end}	3520.8	12142.82	0.29	0.592

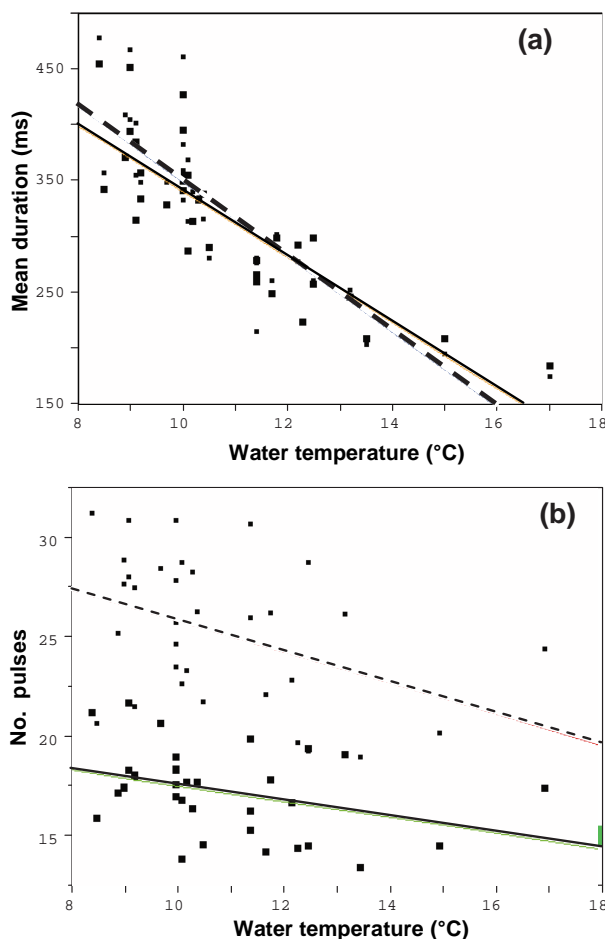


FIG. 2. Effect of water temperature. The dashed regression line and small points are for note A, and the solid line and large points are for note B. (a) linear regression with note duration. Linear fit: note A: Mean Duration (ms) = 686.665 - 33.685 Water Temperature, $R^2 = 0.700$, $P < 0.001$, note B: Mean Duration (ms) = 634.895 - 29.412 Water Temperature, $R^2 = 0.686$, $P < 0.001$. (b) Linear regression with number of pulses per note. Linear fit: note A, Number of Pulses = 33.623 - 0.773 Water Temperature, $R^2 = 0.162$, $P = 0.0182$; note B: Number of Pulses = 21.519 - 0.390 Water Temperature, $R^2 = 0.101$, $P = 0.0376$.

lation between this factor and the number of “B” notes per call in Córdoba.

The fact that none of the call variables measured was

sults obtained in other anuran species, where a negative correlation between male size and call frequency variables has been found, with this being determinant for female choice (e.g. Davies & Halliday, 1978; Márquez, 1995) and even for male-male competition (Bee *et al.*, 1999, 2000). However, this may result from the fact that the carrier frequency of the call has such a wide frequency spectrum. The lack of correlation between male size and call frequency variables in *P. ibericus* suggests that if there are any size-related mating trends in this species, these may result from non-static calling parameters such as call intensity, or from mechanisms of male-male competition (e.g. chorus attendance, or fights).

Since patterns of variation in the acoustic properties

for the same properties (Gerhardt, 1991), and given the similarity of *P. punctatus* and *P. ibericus* mating calls, we should expect, at least in regions of sympatry, that the females base their choice on fine-tuning properties to identify conspecific males. These properties should have a low coefficient of variation, because it is inversely related to various measures of stereotypy (Gerhardt & Davis, 1988).

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