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ADVERTISEMENT CALL OF THE MIDWIFE TOAD FROM THE SIERRAS BÉTICAS *ALYTES DICKHILLENI* ARNTZEN & GARCÍA-PARIS, 1995 (AMPHIBIA, ANURA, DISCOGLOSSIDAE)

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The advertisement calls of the recently described species of midwife toad (*Alytes dickhilleni*) are described, and a characteristic audiospectrogram and waveform of the call are presented. We also provide numerical data about the spectral and temporal features of the calls. Information about calling behaviour and therelationship between call parameters, size, and temperature are provided as well.

INTRODUCTION

Anuran mating calls are important taxonomic determinants due to their role as pre-zygotic species isolating mechanisms. Their analysis has provided valuable material for quantitative comparisons which can be of use to elucidate taxonomic problems such as determining cryptic sister species, (e.g., Blair, 1955, 1958, 1959; Littlejohn, 1959; Littlejohn, Fouquette Jr. & Johnson, 1960; Loftus-Hills & Littlejohn, 1971; Littlejohn & Loftus-Hills, 1968; Gerhardt, 1988; Schneider & Sinsch, 1991; Paillette, Oliveira, Rosa & Crespo, 1992) analyse gradients along hybrid zones, and even test crucial hypotheses in evolutionary biology such as reproductive character displacement or reinforcement (Sanderson, Szymura & Barton, 1992; Littlejohn & Watson, 1985; Littlejohn, 1988). Therefore, since the development of sound analysis techniques, there has been a concerted effort to describe accurately and quantitatively the advertisement calls of most anuran species in tropical areas (e.g., Schlüter, 1979, 1980, 1981; Zimmerman & Bogart, 1984; Márquez, De la Riva & Bosch, 1993, 1995; De la Riva, Márquez & Bosch, 1994; Sánchez-Herraiz, Márquez, Barbadillo & Bosch, 1995; Alcala, Joermann, & Brzoska, 1986; Kuramoto 1986) as well as in the temperate zone (e.g., Schneider, 1966, 1971 1973, 1974; Lörcher, 1969; Paillette, 1967, 1977; Schneider & Sofianidou, 1985; Schneider, Hussein, & Akef, 1986).

In the Iberian Peninsula, two species of midwife toads were previously recognised. The first species, the Iberian midwife toad (*Alytes cisternasii* Boscá, 1879), is endemic to and distributed throughout the south-western third of the peninsula. The second species is the common midwife toad (*A. obstetricans boscai*, Lataste 1879), which is more widely distributed in continental Europe and occupies the northern third of the peninsula and reaches further down along the coasts and on the mountains of central and south-eastern Spain. The populations of midwife toads from the south-eastern mountain ranges (Sierras Béticas and Sub-Béticas) have recently been described as a different species: *Alytes dickhilleni* Arntzen and García-París, 1995. While the vocalisations of the two previously recognized species of midwife toads from the Iberian Peninsula have already been described (Crespo, 1981; Crespo, Oliveira, Rosa, & Paillette, 1989; Márquez & Verrell, 1991), the calls of the new species remain unknown because none of the previous descriptions of the calls included recordings from the distribution area of *A. dickhilleni*. In this paper, we contribute to the knowledge of Spanish herpetofauna by describing the advertisement calls of *A. dickhilleni*. This bioacoustical information complements the description of the new species, which is based on genetic and morphological characters (Arntzen & García-París, 1995).

MATERIAL AND METHODS

Male advertisement calls were recorded in 1992 from populations of *Alytes dickhilleni* in Sierra de Baza (UTM 30SWG13), Sierra Nevada (UTM 30SWG50), Sierra de Cazorla (UTM 30SWG09) (Andalucía), and one population from Sierra de Alcaraz (UTM 30SWH45) (Castilla-La Mancha), all in south-eastern Spain. Recordings were obtained with Sennheiser ME 80 directional microphones and a Sony WM D3 or a Marantz PMD 221 tape recorder. When a male could be observed calling, immediately after recording, its cloacal temperature was measured to the nearest 0.1 °C with a Fluke 52 thermocouple thermometer, and the snout-vent length (SVL) was measured to the nearest mm by pressing the toad flat (ventral side) against a ruler.

Recordings were processed with an Apple Macintosh-based digital signal analysis system. Digitalisation and editing were completed at a sampling frequency of 44.1 kHz. and 16-bit resolution with Sound Tools software and hardware (version 2.5, by Digidesign Inc.). Signalyze software (version 3.12, by Infosignal Inc.) was used to obtain numerical information from audiospectrograms and waveforms. Frequency information was obtained through fast Fourier transform (FFT) (width, 1024 points; frequency resolution, 22 Hz). Given the lack of energy of the harmonics above the fundamental, only three variables were measured: fundamental (= dominant) frequency, call duration, and duration of interval between calls.

RESULTS

Males could be found calling throughout the period of the study (May, June, and July 1992), although they are likely to be active at other times of the year given the developmental stages of the larvae found in the different sites. Like other species of *Alytes* in the Iberian Peninsula (Crespo, 1981; Crespo *et al,*. 1989; Márquez & Verrell, 1991; Márquez, 1995*a*, 1995*b*), males called primarily at night from the open, near their refuges (holes in the ground or under rocks often in banks or near eroded sections of the ground). Males could also call extensively from within their burrows, and in some particularly undisturbed sites, males could be heard during the day calling buried in the ground. Male calling-sites were always in the vicinity of a permanent or near-permanent body of water: mountain springs (natural, or modified by humans), man-made water catchments, troughs, and permanent streams.

A sample of 2 to 17 calls was analysed, comprising 50 males (480 calls total), from four populations. In Table 1 we present, for each population studied, the numerical parameters (mean, standard deviation, and range) of call duration, dominant frequency, and interval between calls. For the sound parameters, means of all the calls obtained from each individual were used. Table 1 also includes the mean recording temperature and the mean snout-vent length (SVL) of the calling individuals, as well as the mean and range of the within-recording coefficients of variation of the three sound parameters.

A characteristic waveform and audiospectrogram of the call are shown in Fig. 1. The calls are extremely simple tonal notes, with most power concentrated in the fundamental (dominant) frequency.

A Kruskal-Wallis Anova test indicated that between-individual variability was larger than within-individual variability for all of the acoustical parameters measured (fundamental frequency, N_{calls}= 480, N_{males} = 50, H = 438.23, P < 0.001; duration, N_{calls}= 444, N_{males} = 50, H = 394.57, P< 0.001; call interval, $N_{call\ intervals}$= 365, N_{males} = 49, H = 157.8, P < 0.001). This result suggests that all parameters could provide information on the calling individual at the particular time of calling. The within-recording coefficients of variation of the three sound parameters are low (< 9 %) suggesting that call duration and dominant frequency would be a "static" (*sensu* Gerhardt, 1991) characteristic of the call, while interval between calls would be a "dynamic" characteristic having high coefficients of variation (> 39 %).

Because the numbers of recordings from most populations taken individually were not sufficient to establish a significant association between sound parameters and male size or temperature, the data from all the populations were used to study the relationships between male size and temperature and call parameters. On the one hand, call dominant (=fundamental) frequency was significantly correlated with male SVL (N = 36, R = 0.46, P < 0.0046, y = -9.1 x + 1772.5) (Fig. 2a.). However, in a multiple regression between male size and temperature vs. call dominant frequency, only male size was significantly correlated (N= 35; SVL, partial F= 4.6, P < 0.0396; temperature, partial

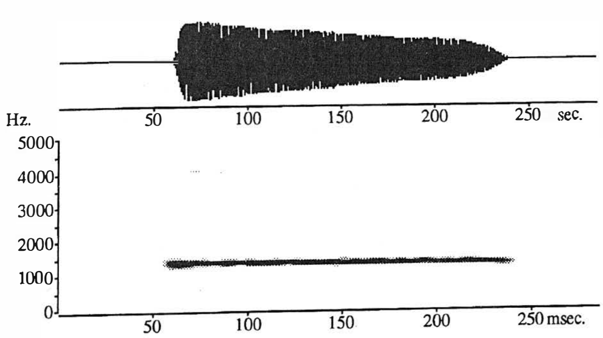

FIG. 1. Waveform (upper) and audiospectrogram (lower) of a characteristic call of a male *Alytes dickhilleni* (male recorded in Sierra de Cazorla, SVL 42 mm, cloacal temperature 9.9°C. Note that the ordinate for the waveform is relative and linear, and therefore a scale is not provided.

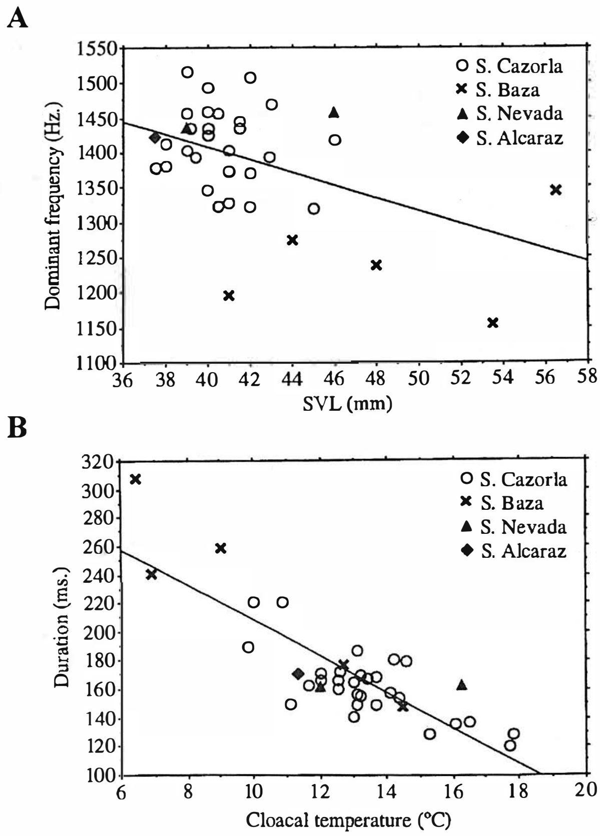

FIG. 2. A: Linear regression of male size (SVL) and call dominant frequency for the recorded individuals captured in the four populations studied. B: Linear regression of cloacal temperature of the animal recorded and call duration of the recorded individuals captured in the four populations studied.

TABLE 1. Mean, standard deviation (in parenthesis), and range, of male size (SVL), cloacal temperature, and three call parameters from the calls of male *Alytes dickhilleni* for each population studied. The data for the acoustical parameters include all animals recorded, while size and cloacal temperature was measured only on those recorded animals that were subsequently captured. The coefficients of variation of the sound parameters included (CV) are within-recording coefficients of variation (expressed as percentages).

Population	Individuals recorded	Individuals captured	SVL (mm)	Cloacal temp. (°C)	Duration (msec.)	Dominant frequency (Hz.)	Interval between calls (msec.)
Sierra de Cazorla	38	31	40.8 (2.2)	13.2 (2.1)	161.0 (24.7)	1404 (61)	5989.9 (1836.1)
			37.5-46.0	9.8-17.8	114.9-220.0	1298-1560	3084.0-10201.2
					CV 4.2	CV 0.8	CV 39.6
					CV_{range} 0.4-14.5	CV_{range} 0.0-3.5	CV_{range} 8.4-93.9
Sierra de Baza	5	5	48.6 (6.4)	9.9 (14.5)	226.4 (64.1)	1241 (73)	5435.2 (2299.8)
			41.0-56.5	6.5-14.5	148.6-307.9	1154-1343	3313.5-8975.0
					CV 8.5	CV 2.9	CV 47.9
					CV_{range} 2.7-18.2	CV_{range} 1.2-7.9	CV_{range} 22.0-74.9
Sierra Nevada	5	2	42.5 (4.9)	14.5 (3.0)	150.0 (28.1)	1371 (125)	4313.6 (2166.8)
			39.0-46.0	12.0-16.3	113.7-179.6	1151-1457	1148.7-6889.64
					CV 5.2	CV 0.5	CV 48.7
					CV_{range} 2.0-7.1	CV_{range} 0.0-1.1	CV_{range} 23.3-83.9
Sierra de Alcaraz	2	1	37.5	11.3	156.5 (20.8)	1364 (83)	4636.6 (37.2)
					141.8-171.2	1305-1422	4610.3-4662.9
					CV 3.5	CV 1.2	CV 61.35
					CV_{range} 2.5-4.4	CV_{range} 0.8-1.6	CV_{range} 36.2-86.5

F= 1.34, $P < 0.26$). On the other hand, call duration was significantly correlated with recording temperature (N = 38, R = 0.836, $P < 0.001$, $y = -12.4\,x + 332$; Fig. 2b.); however, a multiple regression of male size and temperature vs. call duration showed that male size was also significantly correlated with call duration (N= 35; SVL, partial F= 10.58, $P < 0.0027$; temperature, partial F= 45.61, $P < 0.001$). The significance of the relationship between male size and call duration disappears if the five data points from the recordings of individuals from Sierra de Baza are excluded. Therefore, this result has to be taken with caution because the individuals from Sierra de Baza were markedly larger than the rest.

DISCUSSION

The advertisement calls of the other three known species of *Alytes* are short (100-160 ms long), extremely soft, tonal notes repeated at relatively long intervals (0.5 - 10 s) (Crespo, 1981; Crespo *et al.*, 1989; Márquez & Verrell, 1991; Heinzmann, 1970; Bush, 1993).

The range of fundamental frequencies for the four populations of *A. dickilleni* (1151 -1560 Hz.) is wider than that found previously for any single species of continental *Alytes*. Heinzmann (1970) reported that the range of frequencies was 1240-1495 Hz in individuals of *A. o. obstetricans* of different sizes from a population near Tübingen (Germany). Crespo *et al.* (1989) reported a range of 1.25 - 1.47 kHz. for *A. obstetricans boscai* from three populations in northern Portugal. They also reported a range of 1.35 - 1.57 kHz. for *A. cisternasii* from central and southern Portuguese populations. Márquez (1995*a*) reported a range of 1009 - 1373 Hz. for a montane population of *A. obstetricans boscai* from Sierra de Guadarrama, in central Spain and a range of 1362 - 1645 Hz. for a population of *A. cisternasii* from Mérida, in Extremadura, Spain. Moreover, Márquez (1995*b*) found that a montane population of *A. obstetricans* from Formigal, in the Spanish Pyrenean Mountains had a range of 1080-1428 Hz. Similarly, the range of call durations found in our study is large (113.7-220.0 ms.), wider than most ranges reported to date, although the distribution of this parameter may be related to the good correlation between call duration and temperature in *Alytes* (Heinzmann, 1970; Márquez, 1995) and other Discoglossidae or Bombinatoridae (Zweifel, 1959; Schneider *et al.*, 1986).

The two static call characteristics appear to have different roles in the communication of *Alytes*. Given the published information on the calls of other species in the genus, it appears that the most obvious parameter in the frequency domain (dominant or fundamental frequency) plays an important role in sexual selection. In two populations of midwife toads and one of Iberian midwife toads, low-frequency calls (relative to the population average) are more attractive to females than are high-frequency calls (Márquez, 1995*a*, 1995*b*). Alternatively, species identity appears to be more closely related to temporal parameters in the populations of discoglossid frogs studied. Among the different species and subspecies of *Alytes* that occur in the Iberian Peninsula, it appears that call duration relative to temperature is the most effective discriminating parameter (Márquez & Bosch, in prep.). In other related taxa, the study of the advertisement calls of males from two sympatric species of *Bombina* Oken 1816, in central Europe showed that calling rate was the most important discriminating parameter between the species (Lörcher, 1969; Schneider *et al.*, 1986; Sanderson *et al.*, 1992). Curiously enough, the variability obtained in our recordings suggests that call repetition rate is a dynamic characteristic for this species and therefore it could hardly be effective as a species discrimination characteristic. Similarly, in an extensive comparative study of advertisement calls of green frogs, Schneider & Sinsch (1991) found that such temporal parameters (corrected by temperature) were the most important characters for discriminating between major taxonomic groups.

The geographic distribution of *A. dickhilleni* is extremely fragmented. All populations occur on the mountaintops of southeastern Spain, in different ranges isolated by large sections of extremely dry lowland. Moreover, even within a given range, there are often extremely long distances between the nearest locations with permanent or near permanent bodies of water (Márquez, García-París & Tejedo, 1994; Arntzen & García-París, 1995). Therefore, a more detailed comparative study of the bioacoustical characteristics of different populations with different degrees of isolation between them, may prove fruitful for understanding the effect of isolation on a character crucial for species recognition.

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