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Acoustic Repertoire of *Melanophryniscus cupreuscapularis* (Céspedes and Álvarez 2000) (Anura: Bufonidae): Advertisement, Encounter, and Release Calls

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ABSTRACT.—We describe the vocal repertoire of *Melanophryniscus cupreuscapularis*, a bufonid toad of the *Melanophryniscus stelzneri* species group, which has been recently listed as “Vulnerable” by the Asociación Herpetológica Argentina. Recordings were obtained in “Paraje El Perichón,” a relict of *Schinopsis balansae* “quebracho” forest near Corrientes City, Argentina, in September 2007. We provide data on the structure and frequency of three kinds of vocalization: the advertisement, the encounter, and the release calls. We found that the advertisement call is formed by two distinct sections: a first segment consisting of short emissions, usually grouped, and a fast trill. We found that the encounter call is also composed of two segments but that the number of simple emissions in the first segment is greater and more variable (6–60) than in the advertisement call, and the emissions are ungrouped. Although the dominant frequency was similar to the advertisement call, the encounter call frequency was 2,184 Hz (range: 1,832–2,482). The release call is composed of isolated emissions emitted at a lower frequency (1,798 Hz, range: 1,712–1,926) than are the other calls. The structure of the advertisement call was similar to that of other species of the *stelzneri* group.

RESUMEN.—Se describe el repertorio vocal de *Melanophryniscus cupreuscapularis*, un bufónido que pertenece al grupo *stelzneri* recientemente categorizada como “vulnerable” por especialistas de la Asociación Herpetológica Argentina (AHA). Las grabaciones fueron obtenidas en septiembre de 2007 en el Paraje “El Perichón,” un relicto de bosque de quebracho colorado, *Schinopsis balansae*, cercano a la ciudad de Corrientes, Argentina. Proveemos información sobre el análisis estructural y de frecuencia de tres tipos de vocalizaciones: canto de advertencia, de encuentro y de rechazo. *M. cupreuscapularis* emite un canto de advertencia formado por dos secciones diferentes: un primer segmento compuesto de emisiones aisladas, generalmente agrupadas, seguidas por un rápido vibrato. El canto de encuentro está compuesto también por dos segmentos, pero en este caso el número de emisiones simples del primer segmento es mayor y más variable (6–60) que en el canto de advertencia y se presentan desagrupadas. Si bien la frecuencia dominante es similar a la del canto de advertencia, en el canto de encuentro estas ocurren aproximadamente a 2,184 Hz (1,832–2,482). Finalmente, el canto de rechazo es una vocalización conformada por emisiones aisladas emitidas a menor frecuencia (1,798 Hz, range: 1,712–1,926) que en los otros dos casos. Nosotros encontramos similitudes entre la estructura del canto de esta especie con la de otros miembros del grupo *stelzneri*.

The genus *Melanophryniscus* is represented by 25 species, all living in South America (Argentina, Brazil, Bolivia, Paraguay, and Uruguay). *Melanophryniscus cupreuscapularis* is one of the 11 species included in the *Melanophryniscus stelzneri* species group (Cruz and Caramaschi, 2003; Baldo and Basso, 2004; Céspedes and Motte, 2007; Céspedes, 2008; Frost, 2013). Although some studies have described various aspects of the life history of these species (Prigioni and Garrido, 1989; Filipello and Crespo, 1994; Bustos Singer and Gutierrez, 1997; Duré, 2004, 2006; Vaira, 2005; Schaefer, 2007; Cairo et al., 2008; Duré et al., 2009), vocalizations of *M. cupreuscapularis* have not been well documented. *Melanophryniscus cupreuscapularis*, whose range does not exceed 20,000 km², inhabits forests of Quebracho colorado (*Schinopsis balansae*) and Urunday (*Astronium balansae*). This vegetation is characteristic of the northwest triangle of Corrientes province in Argentina and is fragmented attributable to human action. The destruction of its habitat, coupled with other characteristics of the life history of these species, such as low reproductive potential, explosive breeding, and the tendency for a specialized diet (ants), makes this taxon worthy of special attention (Vaira et al., 2012). Information related to the life history of these species will inform conservation decisions and assist in developing conservation strategies.

Our aim was to describe part of the vocal repertoire of *M. cupreuscapularis*, by analyzing spectral and temporal parameters of the advertisement, encounter, and release calls.

MATERIALS AND METHODS

During a breeding event, we surveyed temporary ponds in the type locality of *M. cupreuscapularis*, the Corrientes Redbelly Toad (“El Perichón”; 27°25'55.6"S, 58°44'47.8"W, datum WGS84), located 10 km northeast of Corrientes City, Argentina, to obtain acoustic recordings from breeding males. The data collected for this analysis were obtained from two surveys on 21 and 25 September 2007 following heavy rain. The area is within the Chacoan Domain, Oriental Chaco District (Cabrera and Willink, 1980), and characterized by numerous temporary, semipermanent, and permanent water bodies. The mean annual precipitation is 1,500 mm, and the mean annual temperature is 23°C. According to Carnevali (1994), the original plant formation at the collection site was *Schinopsis balansae* “quebracho” forest, which is currently extremely degraded and largely replaced by sclerophyllous forest, with prevalence of *Prosopis affinis*, *Prosopis nigra*, *Acacia caven*, and *Celtis* spp. and numerous colonies of *Aechmea distichantha* and *Bromelia* spp.

Field Methodology.—All *M. cupreuscapularis* individuals recorded were captured, marked for individual recognition by toe-clipping, and released at the calling site. Prior to release, the following morphometric variables were measured with a digital caliper (ESSEX[®], accuracy 0.01 mm): snout–vent length (SVL), head width (HW) and tibio-tarsus length (TTL). Also, we recorded environmental and behavioral variables: water temperature (°C); air temperature (°C); humidity (%); depth of water body in the calling site (mm); hour of recording; and light intensity at the calling site (measured with a luximeter, MASTECH MS6610). We used an Audio-Technica[®] ATR55 directional microphone and a Panasonic[®] RQ-L31 tape recorder to record calls. The recordings were analyzed using Adobe

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TABLE 1. Call variables from four specimens used for quantitative description of *Melanophryniscus cupreuscapularis* advertisement call from Corrientes, Argentina. Data are shown as mean (minimum and maximum values).

Characteristics	Advertisement call #1	Advertisement call #2	Advertisement call #3	Advertisement call #4
Date	21/09/2007	21/09/2007	25/09/2007	25/09/2007
Number of call analyzed	5	5	5	5
Air temperature (°C)	28.1	22	21.5	18
Water temperature (°C)	27	25.8	20	21
Humidity (%)	55	84	45	36
Light intensity refuge (lux × 100)	154	0.21	70	296
Hour (hhmm)	1625	1733	1650	1710
Call duration (sec)	5.050 (2.42–8.01)	3.023 (1.12–6.66)	5.583 (3.28–7.21)	10.809 (5.39–19.38)
Dominant frequency (Hz)	2,206 (2,045–2,357)	2,270 (2,176–2,357)	2,337 (2,252–2,338)	2,444 (2,148–2,556)
Interval call (sec)	1.95 (1.09–2.98)	2.10 (1.35–2.78)	4.34 (2.88–7.82)	13.30 (4.43–19.31)
First segment duration (sec)	4.70 (2.03–7.69)	2.69 (0.77–6.39)	4.26 (2.72–6.73)	9.74 (4.02–18.15)
Second segment duration (sec)	0.26 (0.23–0.31)	0.23 (0.19–0.27)	0.56 (0.54–0.57)	0.59 (0.51–0.78)
Interval between first and second segment (sec)	0.16 (0.08–0.36)	0.10 (0.04–0.16)	0.10 (0.06–0.13)	0.41 (0.22–0.72)
Number of short notes	12 (9–14) Grouped	10 (6–17) Grouped	12 (8–16) Grouped	17 (11–24)
Short notes duration (sec)	0.04 (0.01–0.05)	0.02 (0.01–0.04)	0.04 (0.02–0.07)	0.034 (0.01–0.06)
Interval between short notes (sec)	0.37 (0.04–2.61)	0.27 (0.09–3.15)	0.36 (0.06–1.75)	0.58 (0.10–7.45)
Pulse number of second segment	25.6 (23–29)	20.4 (16–24)	42 (40–43)	51 (41–71)
Pulse rate of second segment	98.09 (92.35–100.77)	88.95 (96.77–84.03)	74.03 (69.44–75.70)	86.62 (78.24–91.08)

Audition 1.0 and Syrinx 2.6h (Burt, 2006), using FFT with 2,408 points, at a rate of 44100 hz and 16-bit precision. To facilitate the comparison with recordings of other species of the group, we recorded the same variables considered by other authors in previous call studies for the genus *Melanophryniscus* (Baldo and Basso, 2004; Ferrari and Vaira, 2008): dominant frequency (hz); call duration (sec); call interval (sec); first segment duration (sec); second segment duration (sec); interval between first and second segment (sec); number of short notes; duration of short notes; interval between short notes (sec); pulse number of the second segment; and pulse rate of the second segment. The terminology used to describe the calls follows Heyer et al. (1990). Definitions of call types follow Duellman and Trueb (1994), Wells (2007), and Vitt and Caldwell, 2009.

RESULTS

Males of *M. cupreuscapularis* called after rainfall and during the day, from morning to sunset, from vegetation associated with ephemeral ponds and streams.

Advertisement Call.—On 21 September 2007, at 1625 h, we recorded the call of a *M. cupreuscapularis* male (male 1) with SVL = 22.66 mm, HW = 7.48 mm, and TTL = 8.24 mm. The depth of the water body at the site of vocalization was 35 mm. The same day at 1733 h, we recorded another male (male 2), with SVL = 21.24 mm, HW = 7.12 mm, and TTL = 7.58 mm. The depth of the water body at the site of vocalization was 60 mm. On 25 September 2007, we recorded two other males (males 3 and 4). Male 3, with SVL = 25.14 mm, HW = 7.08 mm and TTL = 8.36 mm, was recorded at 1650 h. The depth at the site of vocalization was 10–20 mm. Male 4, with SVL = 22.62 mm, HW = 7.08 mm, and TTL = 7.60 mm, was recorded at 1710 h. The depth at the site of vocalization was 80 mm. All individuals recorded were resting on vegetation, with their body submerged partially in the water. In each case, we analyzed five consecutive calls (Table 1).

The structure of the advertisement call consisted of a compound call with an average dominant frequency of 2,200–2,400 Hz, formed by two clearly distinguished segments. The first segment was formed by short and isolated emissions, highly variable in number (6–24), followed by a short trill (second segment) of 0.19–0.78 sec duration (Table 1). The short

notes of the first segment were variable in number (6–24) and generally were grouped (Fig. 1A). However, it is important to highlight that, in some cases, the first segment showed ungrouped short notes (Fig. 2A,B; Table 1).

Encounter Call.—At 1515 h, on 21 September 2007, we recorded vocalizations emitted by two males while they approached each other and then fought for several seconds. As they fought, both individuals continued vocalizing, bumping each other by inflating their vocal sac, and then wrestling repeatedly. After the fight, the smaller male (male B) left the scene and positioned itself about 3 m away. We captured and measured both individuals: male A: SVL = 24.10 mm, WH = 7.36 mm, and TTL = 8.18 mm; male B: SVL = 22.20 mm, WH = 7.28 mm, and TTL = 8.68 mm. We analyzed the recording obtained during the fight and were able to isolate the calls of one of the individuals, but we could not determine whether the isolated call belonged to male (B) that left the scene or to male (A) (Fig. 1B). The encounter call was also composed of two segments, but, in this case, the number of simple emissions was greater (6–60) than that of the advertisement call and was ungrouped. Although the dominant frequency was similar to the advertisement call, in the encounter call, it occurred at 2,184 Hz (range: 1,832–2,482; Table 2).

Release Call.—At 1545 h, on 21 September 2007, release calls were recorded from male B described in the encounter call. Prior to the release, two males (A and B) were placed in a bucket. The smaller male (B) was then amplexed by the larger male (A) and began producing release calls. The release call was composed of isolated emissions emitted at a frequency of 1,798 Hz (range: 1,712–1,926), lower than calls in the other two cases. The short notes were shorter than those recorded in the first segment of the call of the other two types of vocalizations but exhibited a greater distance between emissions, which, in addition, were more constant (Table 2).

DISCUSSION

Usually, males of *M. cupreuscapularis* can call during April, August, September, and October (months in which we also found gravid females), although not always during all these months in a single year (Schaefer, 2007; EFS and MID, pers. obs.). The recordings analyzed in this study were taken from

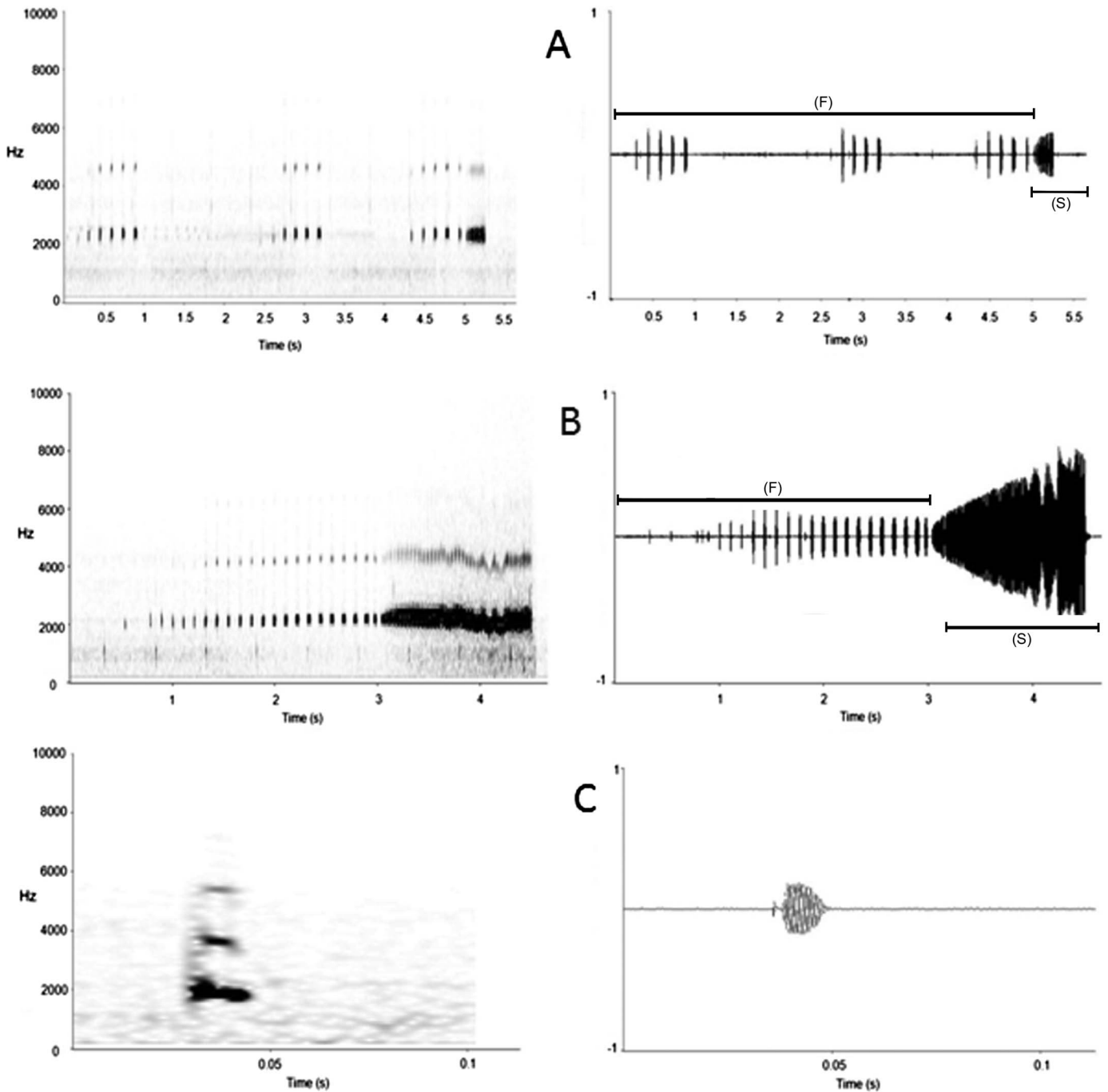


FIG. 1. Sonogram (left) and ocollogram (right) of an advertisement (A), an encounter (B); and a release (C) call of *Melanophryniscus cupreuscapularis* from Corrientes, Argentina. (F): first segment; (S): second segment

two surveys, after torrential rains occurred within the breeding season of this species. During the observations, males and females were present and we observed amplexus, which indicates a relatively synchronous arrival of both sexes at the breeding site (Schaefer, 2007; EFS and MID, pers. obs.), similarly to that observed in other species with very short breeding periods (Wells, 2007). Bioacoustic studies have shown that all the species of the *M. stelzneri* group have an advertisement call composed of short emissions followed by a trill (Barrio, 1964; Kwet and Miranda, 2001; Baldo and Basso, 2004; Kwet et al., 2005; Ferrari and Vaira, 2008). *Melanophryniscus cupreuscapularis* emits an advertisement call formed by two distinct segments: a first segment consisting of a short emission and a second

segment consisting of a fast trill. This feature is consistent with most previous descriptions of the calls of other species in this group, but differences in the group exist. For example, a recent paper (Kurth et al., 2013) describes the courtship call of *Melanophryniscus klappenbachi* from Paraguay recorded in captivity, which exhibits remarkable structural differences from other species in this group (Table 3). Unlike most species of the group, we found that *M. cupreuscapularis* presents a call with a first segment consisting of short notes, which are variable in number and divided into two, three, or more groups. The distance between the short notes within each group is lower than that among groups.

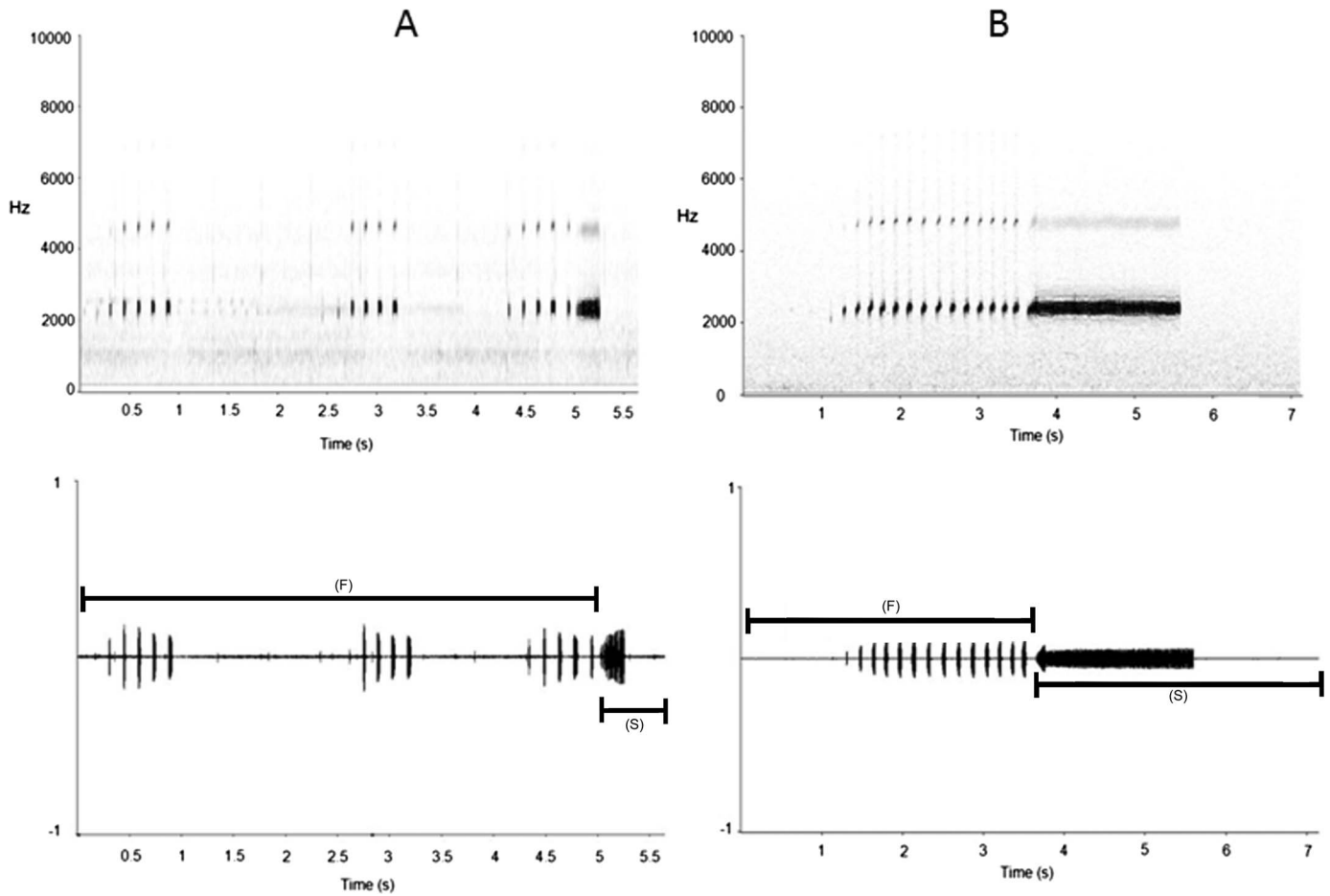


FIG. 2. Sonogram (above) and ocllogram (below) of the two kinds of advertisement calls of *Melanophryniscus cupreuscapularis* from Corrientes, Argentina. A) First segment consists of short notes grouped, and a short trill. B) First segment consists of short notes ungrouped, and a long trill. F: first segment; S: second segment.

A similar structure in the first segment of the advertisement call has been reported by Kwet et al. (2005) for *Melanophryniscus dorsalis* of Laguna, Santa Catarina, Brazil. Also, we found that, in some cases, the first segment had short ungrouped notes. The parameters of the advertisement call of *M. cupreuscapularis* measured in our study were compared with those obtained for other species of the *M. stelzneri* group, including *Melanophryniscus atroluteus* (Baldo and Basso, 2004), *Melanophryniscus*

krauczuki (Kwet and Miranda, 2001), *Melanophryniscus rubriventris* (Ferrari and Vaira, 2008), *M. dorsalis* and *Melanophryniscus montevidensis* (Kwet et al., 2005) and *M. stelzneri* (Barrio, 1964) (Table 3). We found that the duration of the advertisement call (1.12–6.66 sec) is similar to that of *M. stelzneri* (3.2 sec [as described in Barrio, 1964]), *M. rubriventris* (2.13–3.15 sec), *M. montevidensis* (2.2–6.5 sec), *M. dorsalis* (5.5–6.7 sec); a little shorter than that of *M. atroluteus* (Rio grande do Sul, Brazil: 3.4–

TABLE 2. Mean data (minimum and maximum values) of parameters of encounter and release calls of *Melanophryniscus cupreuscapularis* from Corrientes, Argentina. (A) = air temperature; (W) = water temperature

Characteristics	Encounter call	Release call
Temperature (°C)	32(A), 28(W)	32 (A) 28(W)
Hour (hhmm)	1515	1545
Call duration (sec)	10.15 (2.05–25.69)	–
Number of call analyzed	4	15
Dominant frequency (Hz)	2,184 (1,832–2,482)	1,798 (1,712–1,926)
Interval call (sec)	2.98 (1.97–4.28)	0.48 (0.12–1.34)
First segment duration (sec)	7.95 (0.47–22.23)	–
Second segment duration (sec)	1.66 (1.47–1.94)	–
Interval between first and second segment (sec)	0.04 (0.03–0.04)	–
Number of short notes	27 (6–60) (often ungrouped)	–
Short notes duration (sec)	0.03 (0.01–0.05)	0.013 (0.02–0.007)
Interval between short notes (sec)	0.26 (0.02–3.08)	–
Pulse number of second segment	190.71 (170–224)	–
Pulse rate of second segment	114.91 (112.13–118.36)	–

TABLE 3. Average, maximum, and minimum data (in parentheses) of parameters of advertisement calls of seven species of *Melanophryniscus stelzneri* group. *Melanophryniscus cupreuscapularis* data correspond to the male number 2, and data from the other species were collected from literature. *Ma*: *Melanophryniscus atroluteus*, *Mkr*: *Melanophryniscus krauckzaki*, *Mr*: *Melanophryniscus rubriventris*, *Md*: *Melanophryniscus dorsalis*, *Mim*: *Melanophryniscus montevidensis*, *Ms*: *M. stelzneri*, *Mc*: *M. cupreuscapularis*, and *Mki*: *Melanophryniscus klappenbachii*. (a) air temperature; (w) water temperature; FH: fundamental harmonic; 2H: second harmonic; 3H third harmonic. *M.kl** = described as courtship call (specimens in captivity). B: Barrio, 1964; KandM: Kwet and Miranda, 2001.

	<i>M. a</i>	<i>M. kr</i>	<i>M. r</i>	<i>M. d</i>	<i>M. m</i>	<i>M. s</i>	<i>M. c</i>	<i>M. kl*</i>
Call analyzed	12	5	12	3	14	N/D	5	11
Temperature (°C)	17(w)	17(w)	19(a)	20(a)	24(a)	20.5(a,B)	22(a), 25.8(w)	22-24 (a)
Dominant frequency (Hz)	3,000	3,300	1 segment 1,788 (1,704-1,846)	2,600-3,200	2,100-2,800	FH (2,200-2,600)	2,270 (2,176-2,357)	1,900
Call duration (sec)	7.523 (5.09-10.35)	32.699 (25.013-36.646)	2.71 (1.40-3.25)	-	-	2 H (4,600-5,200)	3.023 (1.12-6.66)	0.507 (0.292-0.652)
Interval call (sec)	8.489 (2.922-7.954)	21.988 (18.196-25.737)	1.912 (0.296-1.489)	3.70 (3.5-4.10)	1.98 (1.00-4.5)	3H 7,000-7,600 (B)	2.10 (1.35-2.78)	-
First segment duration (sec)	4.143 (2.575-5.36)	2.031 (1.128-3.16)	0.912 (0.296-1.489)	2.37 (2.0-2.6)	1.58 (1.2-2.0)	1,600-2,600 (KandM)	2.688 (0.78-6.39)	-
Second segment duration (sec)	3.012 (1.832-4.303)	30.4548 (23.784-33.408)	1.365 (0.691-1.648)	2.37 (2.0-2.6)	1.58 (1.2-2.0)	0.5(B) 2.1 (1.8-2.4) (KandM)	0.23 (0.19-0.27)	-
Interval between first and second segment (sec)	0.026 (0.003-0.079)	0.258 (0.085-0.827)	0.435 (0.212-0.929)	-	-	7.3 (4.5-9.3) (KandM)	0.10 (0.04-0.16)	-
Number of short notes (sec)	20.6 (15-25)	8.6 (6-12)	3.8 (2-7)	19 (18-20)	17 (7-28)	26 (12-36) (KandM)	10 (6-17) (grouped)	3-4
Short notes duration (sec)	0.102 (0.006-0.174)	0.009 (0.005-0.023)	0.039 (0.042 -0.048)	0.054 (0.030-0.065)	0.031 (0.02-0.04)	0.11(B)	0.02 (0.01-0.04)	-
Interval between short notes (sec)	0.091 (0.006-0.229)	0.217 (0.147-0.837)	0.285 (0.209-0.496)	0.153 (0.130-0.190)	0.103 (0.078-0.130)	0.09 (B) 0.110-0.130 (KandM)	0.27 (0.09-3.15)	-
Pulse number of second segment	222.38 (139-321)	1298.5 (1018-1,502)	-	161 (152-173)	147 (100-192)	70(B) 65-85 (KandM)	20.4 (16-24)	43 (26-53)
Pulse rate of second segment	75.44 (74.31-76.8)	43.67 (42.35-44.95)	54 (51-58)	62-74	85-95	Barrio, 1964 (B)	88.95 (96.77-84.03)	86 (80.34-88.91)
References	Basso, 2004	Baldo and Basso, 2004	Ferrari and Vaira, 2008	Kwet et al., 2005	Kwet et al., 2005	and Miranda, 2001	Present work	Kurth et al., 2013

4.2 sec; Misiones, Argentina: 5.09–10.35 sec); and remarkably shorter than that of *M. krauczuki* (25.01–36.64 sec).

Also, we found that the dominant frequency 2,270 (2,176–2,357) is a little higher than that reported for *M. rubriventris* (1,687–1,841 Hz), similar to that of *M. stelzneri* (2,200–2,600 Hz) and *M. montevidensis* (2,100–2,800 Hz), and lower than that of *M. atroluteus* (Rio Grande do Sul, Brazil: 1,900–3,100 Hz; Misiones, Argentina: 3,000 Hz), *M. krauczuki* (3,000 Hz), and *M. dorsalis* (2,280–3,200 Hz), which reach and even exceed 3,000 Hz. Finally, although calls of *M. klappenbachi* have been described recently (Kurth et al., 2013), these were not examined in the present work because they were given in a social context different from that of typical advertisement calls.

Wells (2007) and Duellman and Trueb (1994) mentioned that aggressive calls are often variations of the advertisement call. Thus, in many species of anurans, vocalizations have similar dominant frequency but different structure. Wells (2007) argued that, because the aggressive calls do not play a fundamental role in recognition between individuals of the same species, it might be expected that these calls will be less stereotyped than are advertisement calls. Our recordings of encounter calls showed great variability, even when considering individual vocalizations. This was observed in the temporal features, such as the pulse repetition rate and pulse number, as well in the number and arrangement (grouping) of short notes of the first segment of the call. Both males were fighting and presumably had reduced control over expiration and vocalization, which could have contributed to the variability in the temporal characteristics of the calls.

In anurans, release calls usually consist of a series of rapidly repeated broad spectrum notes and are emitted either by males when clasped by another male, or by females when they are not receptive at the time of amplexus, usually accompanied with body vibrations (Wells, 2007; Vitt and Caldwell, 2009). These results are consistent with those reported by Gerhardt (1994), who proposed that release calls are short in duration, are emitted at irregular intervals, and have a variable structure completely different from that of advertisement calls.

Because *M. cupreuscapularis* exhibits an explosive reproductive behavior, with a high number of males concentrated in small temporary pools, it was very difficult for us to obtain isolated recordings of advertisement calls. The high variability observed in advertisement calls may be attributable to its dual role in mating and territoriality.

In summary, we described the vocal repertoire of *M. cupreuscapularis*, a species for which little is known about its vocalizations. We believe that to understand precisely how the acoustic niche is compartmentalized under great pressure of space and time during the reproductive event, it would be useful to obtain more recordings from more individuals under different social contexts.

It is important to note that *M. cupreuscapularis* is listed as “Near Threatened” by the IUCN (2013) and recently listed as “Vulnerable” in the last evaluation of the conservation status of the herpetofauna of Argentina, performed by the Asociación Herpetológica Argentina (AHA) (Vaira et al., 2012). The information provided in this study adds to the small body of literature on the natural history of this species of conservation concern.

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