Advertisement Calls and Interspecific Variation in *Hypsiboas cordobae* and *Hypsiboas pulchellus* (Anura, Hylidae) from Central Argentina

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Abstract: The work aims to expand knowledge of the advertisement calls of *Hypsiboas pulchellus* and *Hypsiboas cordobae* from central Argentina. We provide information on each species’ calling behaviour, and spectral and temporal features of the advertisement call. Both species presented a characteristic vocalization that allows their taxonomic identification. Discriminant analyses showed differences between the two species in study, with erroneous classification percentages lower than 20% (3.45% for *H. cordobae* and 2.5% for *H. pulchellus*). It could be inferred then that there is low intraspecific variability and high interspecific variation in calls.

Key words: bioacoustics, advertisement call, *Hypsiboas cordobae*, *Hypsiboas pulchellus*, Argentina

Introduction

Acoustic communication is an important feature of anuran social behaviour (Schneider, Sinsch 1992, Pargana et al. 2003; Friedl, Klump 2001) and plays an important role in reproductive behaviour (Brenowitz, Rose 1999). In most taxa, males produce advertisement calls to attract conspecific females (Barrio 1964, Brenowitz, Rose 1999, Bosch, de la Riva 2004, Friedl, Klump 2005). These calls constitute the major pre-mating reproductive isolating mechanism in anurans (Gerhardt 1974, Blair 1958). In consequence, the study of vocalization is extensively used to elucidate taxonomic and phylogenetic problems. It is also considered an important character for species identification (Blair 1958, Basso, Basso 1987, Penna, Veloso 1987, Vasara et al. 1991, Schneider et al. 1993, Marquez et al. 1993, Marquez 1995, Di Tada et al. 1996a, 1996b, Kohler, Lotters 1998, Salas et al. 1998, Bosch et al. 1999, Guimarães, Bastos 2003, Pombal, Bastos 2003, Bernal et al. 2004).

Variation in the advertisement call of a particular 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 (De la Riva et al. 1996). Environmental conditions may affect anuran call characteristics (Brenowitz 1986, Bosch, de la Riva 2004) and because of their ectothermic condition, it is expected that the acoustic properties vary with temperature and other climatic variables (Duellman, Trueb 1986). Several studies have demonstrated that environmental factors affect the calls (Vasara et al. 1991, Heyer 1994, Martino, Sinsch 2002, Bosch, De la Riva 2004, Bionda et al. 2006, Baraquet et al. 2007, Bionda et al. 2008).

The genus *Hypsiboas* Wagler, 1830 contains 84 species, most of which are included in seven species groups (Frost 2011, Lehr et al. 2011). One of them is the *Hypsiboas pulchellus* group, which currently contains 36 species (Frost 2011, Köhler et al. 2010, Lehr et al. 2010, Lehr et al. 2011) including *Hypsiboas pulchellus* (Duméril, Bibron, 1841) and *Hypsiboas cordobae* (Barrio 1965) which are known to inhabit the central area of Argentina.

*H. pulchellus*, is a widely distributed amphibien...

Previous studies have addressed spectral and temporal properties of the advertisement calls for these two sister species, and observed differences in advertisement call structure and morphometrics for both species studied (Barrio 1962, 1965, Cei 1980, Basso, Basso 1987, di Tada *et al.* 1996a, Salas *et al.* 1996, Faiovich *et al.* 2004, Baraquet *et al.* 2007). Yet these works are only descriptive, and only the work of Baraquet *et al.* (2007) quantitatively explored the effect of temperature on different call variables. It was therefore necessary to conduct a sampling of advertisement calls at a wide range of temperatures for the application of multivariate statistics to differentiate the species. Also, The IUCN Red List of Threatened Species reports *H. cordobae* as data deficient since it has only recently been removed from synonymy, and there is still very little information on its extent of occurrence, status and ecological requirements, making this species important for study.

In this work we described the spectral and temporal features of the advertisement calls of *H. cordobae* and *H. pulchellus* inhabiting the central area of Argentina. We assessed the temperature influence on several acoustic variables and analysed acoustic differences between both species.

**Material and Methods**

Advertisement call recordings for *H. cordobae* were obtained in several localities of Córdoba and San Luis provinces and advertisement call recordings for *H. pulchellus* were obtained in three localities of Córdoba province (Table 1), the species being allopatric in all localities.

The fieldwork was conducted during both species reproductive period (from September to May) between 2006 and 2010. The advertisement calls were recorded with a Digital Walkman Audio Tape (DAT) Sony® TCD-100 with stereo microphone ECM-MS907 Sony® and TDK™ DAT-RGX 60, at a sampling frequency rate of 44.1 kHz; and air temperature at each calling site was recorded with a digital thermometer (precision = 0.1°C).

The calls were analyzed using Adobe® Audition™ 1.0 (FFT (Fast Fourier transform): 1024 points, 44.1 KHz, resolution 16 bit).

We analyzed 1090 calls from 58 individuals of *H. cordobae* and 1056 calls from 40 individuals of *H. pulchellus*. Each call was characterized by the following parameters: 1. number of notes per call (N/C); 2. call duration (CD); 3. note duration (ND); 4. inter–note interval (IN); 5. inter–call interval (IC); 6. call rate (CR) in calls by minutes (c/m), 7. dominant frequency of the call (DFC), 8. dominant frequency of the note (DFN). Each of these selected calls was filtered to eliminate noise.

We calculated mean, standard deviation, maximum and minimum values for each variable using Statgraphics Plus 5.0. All data were tested for normality of distribution using the Shapiro-Wilks test. The species were compared using the variables shared by all individuals (CD, ND1, ND2, IN1, CR, DFC, DF1, DF2) with parametric one–way ANOVA.

To examine the relationships among advertisement call and temperature we used the residuals from the regression of call variables on air temperature. All variables that were significantly correlated with temperature were standardized to 18°C before further analyses. The temperature standardized call data was analyzed using discriminant analysis. These analyses were performed using Statgraphics Plus 5.0.

### Results

**Characterization of advertisement calls**

A simple basic advertisement call consisting of two–five tonal notes was observed. The final notes of the calls showed longer duration than the first ones.

Both species emitted their calls forming chorus, although some individuals were found vocalizing alone. The call is commonly initiated by a single individual, and others response to it.

The inter–call interval was longer at the beginning and the end of the calls, as well as during individual calling compared with vocalisation in chorus. Therefore, the inter–call interval had a very high standard deviation, because individuals emitted series of calls separated by short intervals, while the series were separated by longer intervals.
In both species we observed the presence of a simple tonal sound that was alternated with calls. **Hypsiboas cordobae**

Specimens of this species were observed vocalizing at water bodies edges (rivers, streams), on stones or vegetation (on aquatic plants or higher plants, one meter tall). The individuals partly submerged in water among aquatic vegetation were also observed calling.

The results showed three types of advertisement calls consisting of three, four and five notes, respectively. The individuals alternated different types of calls, of which the following can be distinguished: 1. individuals calling with only four notes; 2. individuals calling with four notes alternating with five–note calls; 3. individuals calling with four notes alternating with three–note calls; 4. individuals calling with three, four and five notes alternated (Fig. 1, A); and 5. individuals showing only three notes per call.

In percentages, we found 65.23% (711 calls) of four–note calls, 22.57% (246 calls) of three–note calls, 10.46% (114 calls) of five–note calls, and 1.74% (19 calls) of two–note calls.

From the 60 individuals studied, 21 individuals presented calls of only four notes and only a single individual presented calls of three notes. There were 19 individuals that presented four–note calls alternating with three–note calls, and six individuals showed calls of four notes alternating with five–note calls. There were also 13 individuals that presented alternating calls of three, four and five notes. Finally, eight individuals presented calls of two notes: five of them alternated calls of two, three, four and five notes, and the other three alternated calls of two, three and four notes.

The three types of calls differed in duration. The final notes were longer than the others, that had a similar average duration (Table 2). In some cases, however, the final notes presented a similar or lower duration than the others.

The calls had a dominant frequency between 1160 and 2450 Hz with a slight increase from the first to the last dominant frequency of each note.

The average duration of inter–call interval was 1310 ± 1252.5 ms, with a range of 146–9748 ms. There was a high variability in this parameter. This was also observed in the call rate (CR), which average was 30.8 c/m (10–81 c/m).

We analyzed 63 simple tonal sounds from 20 individuals, with an average duration of 124.52 ms (182–543), and a dominant frequency within the range of 802.31–2196 Hz. The duration of these sounds was greater than the duration of call notes from the same individual, but the frequencies were always lower. **Hypsiboas pulchellus**

Specimens of this species were also observed vocalizing at the edge of water bodies (rivers or gaps), on stones or on vegetation (aquatic plants or higher plants, one meter tall). The individuals of this...
species were observed calling within gaps, on vegetation up to 20 cm from the base, or on aquatic vegetation in contact with water. In this species, there were no records of calls of individuals partly submerged in water.

The results obtained revealed one basic advertisement call formed by two notes (Fig. 1, B). We also found individuals with one–note calls alternating with two–note calls, but in very low proportion, only 1.99% (21 calls out of 1056 analyzed).

As in *H. cordobae*, the final notes of *H. pulchellus* were longer; the second note was longer than the first one (Table 3). The calls had a dominant frequency between 1503 and 2756 Hz, with an average frequency of 2331.99 Hz. The dominant frequency of the second note (2345.24 ± 207.013 Hz) was greater than the dominant frequency of the first one (2212.89 ± 273.287 Hz).

The average value of inter–call interval was 291.33 ± 136.28 ms, with a range of 154–504 ms. The call rate presented a range of 34–211 c/m, with an average of 95.08 c/m.

We analyzed 16 simple tonal sounds from 11 individuals, with an average duration of 71.38 ms (43–105 ms), and a dominant frequency between 1392.2 and 2300 Hz. The duration of these sounds was greater than the call notes from the same individual, but the frequencies were always lower.

### Acoustic differences between *H. cordobae* and *H. pulchellus*.

The most remarkable difference between both species was the number of notes per call, *H. cordobae* presented calls composed by two, three, four and five notes, whereas *H. pulchellus* presented calls composed by only two notes (Fig. 2).

In both species the call types differed in duration, but the final notes were longer than the first ones. However, some individuals of *H. cordobae* presented similar or lower duration in the final notes.

The two–note call of *H. cordobae* was the most similar to the call of *H. pulchellus*. However, the dominant frequency average values were higher for *H. pulchellus* than for *H. cordobae*.

All of them showed significant differences ($p < 0.05$) when compared via one–way ANOVA (CD $p = 0.0000$, $F = 108.29$; ND1 $p = 0.0006$, $F = 12.64$; $p = 0.0000$, $F = 12.64$).

<table>
<thead>
<tr>
<th>Variables</th>
<th>Calls of two notes</th>
<th>Calls of three notes</th>
<th>Calls of four notes</th>
<th>Calls of five notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>116.68 ± 16.02</td>
<td>206.03 ± 34.15</td>
<td>309.94 ± 46.09</td>
<td>354.43 ± 47.74</td>
</tr>
<tr>
<td>ND1</td>
<td>17.32 ± 4.97</td>
<td>24.43 ± 11.99</td>
<td>26.91 ± 10.10</td>
<td>20.50 ± 7.48</td>
</tr>
<tr>
<td>IN1</td>
<td>54.58 ± 12.24</td>
<td>52.89 ± 17.85</td>
<td>66.91 ± 17.76</td>
<td>61.15 ± 12.17</td>
</tr>
<tr>
<td>ND2</td>
<td>42.42 ± 10.83</td>
<td>26.09 ± 11.56</td>
<td>25.12 ± 9.89</td>
<td>20.67 ± 5.44</td>
</tr>
<tr>
<td>IN2</td>
<td>48.72 ± 14.85</td>
<td>57.03 ± 16.17</td>
<td>53.88 ± 11.22</td>
<td></td>
</tr>
<tr>
<td>ND3</td>
<td>50.26 ± 10.82</td>
<td>27.83 ± 10.63</td>
<td>23.39 ± 6.92</td>
<td></td>
</tr>
<tr>
<td>IN3</td>
<td>51.03 ± 15.65</td>
<td>51.81 ± 10.92</td>
<td>27.34 ± 9.50</td>
<td></td>
</tr>
<tr>
<td>ND4</td>
<td></td>
<td></td>
<td>48.87± 10.37</td>
<td></td>
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<tr>
<td>ND5</td>
<td>46 ± 9.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DFC</td>
<td>1911.92 ± 271.45</td>
<td>1812.68 ± 258.30</td>
<td>1865.10 ± 259.13</td>
<td>1691 ± 308.87</td>
</tr>
<tr>
<td>DF1</td>
<td>1830.76 ± 253.96</td>
<td>1728.36 ± 256.55</td>
<td>1769.18 ± 219.21</td>
<td>1563.60 ± 264.85</td>
</tr>
<tr>
<td>DF2</td>
<td>1944.88 ± 274.90</td>
<td>1784.03 ± 242.55</td>
<td>1800.57 ± 230.43</td>
<td>1590.03 ± 278.42</td>
</tr>
<tr>
<td>DF3</td>
<td>1855.4 ± 242.66</td>
<td>1844.12 ± 244.97</td>
<td>1648.65 ± 295.47</td>
<td></td>
</tr>
<tr>
<td>DF4</td>
<td>1888.42 ± 253.51</td>
<td>1693.64 ± 300.79</td>
<td>1726.38 ± 291.22</td>
<td></td>
</tr>
<tr>
<td>DF5</td>
<td></td>
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</tbody>
</table>

![Oscillogram and sonogram](image-url)
Table 3. Mean and SD values of the acoustic variables analysed for the types of call in *H. pulchellus*

<table>
<thead>
<tr>
<th>Variables</th>
<th>Calls of one note</th>
<th>Calls of two notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD</td>
<td>38.62 ± 12.86</td>
<td>132.91 ± 31.47</td>
</tr>
<tr>
<td>ND1</td>
<td>16.59 ± 4.96</td>
<td></td>
</tr>
<tr>
<td>IN1</td>
<td>77.26 ± 31.07</td>
<td></td>
</tr>
<tr>
<td>ND2</td>
<td>37.32 ± 6.85</td>
<td></td>
</tr>
<tr>
<td>DFC</td>
<td>2162.22 ± 305.02</td>
<td>2331.99 ± 213.94</td>
</tr>
<tr>
<td>DF1</td>
<td>2212.89 ± 273.29</td>
<td></td>
</tr>
<tr>
<td>DF2</td>
<td>2345.24 ± 207.01</td>
<td></td>
</tr>
</tbody>
</table>

ND2 \( p = 0.0000, \ F = 50.96 \); IN1 \( p = 0.0000, \ F = 31.26 \); CR \( p = 0.0000, \ F = 38.79 \); DFC \( p = 0.0000, \ F = 48.08 \); DFN1 \( p = 0.0000, \ F = 55.34 \); DFN2 \( p = 0.0000, \ F = 80.64 \).

The simple regression analyses showed that all variables vary with temperature (CD: \( r = -0.623553, \ R^2 = 38.88\% , \ p = 0.0000; \ ND1: \ r = -0.510688, \ R^2 = 26.08\% , \ p = 0.0000; \ ND2: \ r = 0.226563, \ R^2 = 5.13\% , \ p = 0.0241; \ IN1: \ r = -0.289896, \ R^2 = 8.40\% , \ p = 0.0036; \ CR: \ r = 0.561101, \ R^2 = 31.48\% , \ p = 0.0000; \ DFC: \ r = 0.298911, \ R^2 = 8.93\% , \ p = 0.028; \ DF1: \ r = 0.264205, \ R^2 = 6.98\% , \ p = 0.0082; \ DF2: \ r = 0.364726, \ R^2 = 12.40\% , \ p = 0.0002). \)

The discriminant analysis yielded one function with an eigenvalue of 2.53 that explained 100% of the observed variation (\( p = 0.0000 \)). The canonical correlation of 0.85 (close to 1) indicated that the function had a high weight, while the Wilks Lambda of 0.28 (close to 0) indicated that the two selected variables (CD and IN1) were appropriate to discriminate species (Fig. 3). The percentage of cases correctly classified was 96.94% (96.55% for *H. cordobae* and 97.50% for *H. pulchellus*).

**Discussion**

Several authors have already observed differences in advertisement call structure and morphometrics for both species studied, *H. pulchellus* and *H. cordobae* (Barrio 1965, BASSO 1987, Di TADA et al. 1996a, FAIVOVICH et al. 2004). However, there were no previous attempts to quantitatively explore the effect of temperature on different call related variables, and both species were never compared by multivariate statistical methods.

The results of this study coincided with previous descriptions of advertisement calls in *H. pulchellus* and *H. cordobae*, which were characterized as basic tonal calls formed by two to five series of notes, with the final ones of longer duration. Besides, the individuals of both species emitted their calls individually or forming choirs (Barrio 1962, 1965, CEI 1980, BASSO, BASSO 1987, Di TADA et al. 1996a, SALAS et al. 1998).

The individuals of both species were observed calling out of water, on vegetation or rocks. The individuals of *H. cordobae* were also observed calling partially submerged in water among aquatic vegetation. This behaviour was also described by Barrio (1965) and Gallardo (1987).

Moreover, both species are characterized by the emission of a single sound, different from the typical call notes. This sound (Barrio 1962, 1965) is emitted just once or several times when the individual begins its call. However, we observed that this note was also performed between calls or at the end of calls. This sound has duration of 130 ms (Barrio 1962), which is consistent with our results for *H. cordobae* (124.52 ms), but it is higher than the mean duration we observed for *H. pulchellus* (71.38 ms).

We distinguished three different calls for *H. cordobae* composed of three, four and five notes per call, and only one call formed by two notes for *H. pulchellus*. Both species calls differed significantly in...
duration and dominant frequency. Our results are in agreement with several authors’ description of both *H. pulchellus* (BARRIO 1962, 1965, CEI 1980, GARCÍA et al. 2003, BARAQUET et al. 2007) and *H. cordobae* advertisement calls (BARRIO 1965, CEI 1980, DI TADA et al. 1996a). However, BASSO, BASSO (1987) indicated that the call of *H. pulchellus* consists of four notes distributed in two groups. Our results do not show that structure, we basically found a two–note call in this species. Both BARRIO (1965) and DI TADA et al. (1996a) observed calls of three and four notes for *H. cordobae*, while in this work we also found calls with five notes.

Calls comprised of one note for *H. pulchellus* and of two notes for *H. cordobae*, which differ from the basic pattern for both species, are consistent with reports by other authors indicating that call can be modified in different ways (BARRIO 1965, VASARA et al. 1991, MARQUEZ et al. 1993, BARAQUET et al. 2007).

In both species, the final note was the longest and the three–note call of *H. cordobae* was the most similar to that of *H. pulchellus*.

The bioacoustic variables of the advertisement call in *H. pulchellus* (call duration, duration of the first note, duration of the second note and dominant frequency of the call) were similar to those present–
ed by BARRIO (1965) and DI TADA et al. (1996a). However, the duration of the third and fourth notes as well as the dominant frequency range reported by those authors were lower compared to values obtained in this work.

The dominant frequency of the call and that of each note were higher in *H. pulchellus* in comparison with *H. cordobae*. This difference is consistent with differences in body size between species; *H. cordobae* is slightly larger than *H. pulchellus* (CEI 1980, GALLARDO 1987). The frequency of anuran acoustic signals is partially determined by the shape and mass of the laryngeal apparatus, which is in turn related to body size (DUELLMAN, TRUEB 1986). Therefore, an increase in the vocal cords cartilage and in the soundboard will produce deeper and lower frequency sounds (BERNAL et al. 2004).


Intra and interspecific variation was observed in inter–note interval and call rate. Several authors have shown that male frogs can change some characteristics of their call to reflect the social context within which they are calling (SCHWARTZ 1989, BURMEISTER et al. 1999, BERNAL et al. 2004), and that the most common response of males to other competing males is an increase in the call rate. Thus, when individuals call alone, especially when they begin to vocalize, the call rate is low, whereas when they call in chorus the call rate increases significantly (SCHWARTZ, WELLS 1985, BOSCH et al. 2000, BOSCH, de LA RIVA 2004, BERNAL et al. 2004, BERNAL et al. 2007). BERNAL et al. (2004) attributed these variations to a strategy of males to avoid overlap and interferences of the calls, and thus, females consistently select the alternative with high call repetition rate (SCHWARTZ, WELLS 1985, BOSCH, MARQUEZ 2005). This could explain the fact that in both species the inter–call interval was longer at the beginning and at the end of their calls, as well as during individual calling compared with vocalisation in chorus.

However, other factors seem to affect all these acoustic parameters. ZIEGLER et al. (2011) identified a plausible causal structure connecting environment, individual attributes, and temporal and spectral adjustments as direct or indirect determinants of the observed variation in call attributes of *Hypsiboas pulchellus*. These authors revealed a strong effect of the habitat structure on the temporal parameters of the call, and suggested the effects of local vegetation, temperature and proportion of water in the environment as determinants of the temporal variables. In addition, the mentioned authors showed that the spectral variables were related only to the size of the calling males, and explained this as an effect of the site temperature that determined the size of organisms calling at each site and thus indirectly affecting the dominant frequency of the call. In summary, the two main determinants of the call attributes: On the one hand, environmental conditions consistently affect temporal attributes, and on the other hand, individual attributes affect the spectral structure of calls; the males of *H. pulchellus* have the potential to adjust their calls in response to their local environment.

The discriminant analysis allowed us to differentiate the two species with a highly significant function. The percentage of the correctly classified cases was high in both species. SCHNEIDER, SINSCH (1992) considered these classification percentages as a quantitative measure for the local variability in advertisement calls, which can be distinguished from taxonomic differences among populations. In addi-
tion, they stated that erroneous classification rates below 20% were evidence of random variation. We obtained classification percentages lower than 20% (3.45% and 2.5% for *H. cordobae* and *H. pulchellus*, respectively). We could say then that there is an evidently low intraspecific variability and a high interspecific variation in the advertisement calls.

The results obtained here show that the two species have common features, thus reflecting their phylogenetic proximity (Faiovich et al. 2004, Faiovich et al. 2005). However, because the anuran call is an effective mechanism for pre-reproductive isolation and is therefore useful for species identification (Duellman, Trueb 1986, Di Tada et al. 1996a), our results also show differences in the advertisement call of both species.

Its characterization and variability, coupled with the many factors that may affect it are then valuable information for taxonomic, phylogenetic and ecological approaches.

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