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Differential and additive effects of natural biotic and anthropogenic noise on call properties of *Odontophrynus americanus* (Anura, Odontophryinidae): Implications for the conservation of anurans inhabiting noisy environments

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ABSTRACT

Successful reproduction in most anurans is associated to acoustic communication and negative effects of auditory masking by natural biotic and anthropogenic noise are known in this group. However, the potential additive effect of both noise types has been scarcely studied. We examine *in situ* the acoustic variation of 135 males (n = 975 calls) of *Odontophrynus americanus* in areas from Central Argentina with (TN) and without traffic noise (N-TN) and in presence (Ch) or absence (N-Ch) of chorus. The effect of noise condition on four call properties (call duration [CD], intercall interval [ICI], pulse rate [PR] and dominant frequency [DF]) were analyzed using linear mixed models (LMM and GLMM). A Principal Component Analysis (PCA) was performed to identify the acoustical properties that best accounted for variation among all possible noise conditions (N-TN/N-Ch; N-TN/Ch; TN/N-Ch and TN/Ch). PR and DF showed significant higher values in TN than N-TN sites while a significant increase in ICI and a decrease in DF were found in chorus situation. Analyzing combined effects, PR significantly increased in N-TN/N-Ch condition and decreased under TN/Ch. ICI was significantly higher in N-TN/N-Ch condition. PCA showed a separation of males calling in noisy condition mainly associated to higher values of DF and CD. The acoustic variation observed in noisy environments could have implications in the reproductive strategies of the individuals although new studies should be conducted to analyze the effect of this variation on the recognition and choice by females. Our outcomes highlight the importance of conducting *in situ* behavioral studies considering the additive effects of different environmental noise sources.

1. Introduction

In the last two decades the concern of conservation biologists about the possible effects of noise on animal acoustic communication systems has increased (Velez et al., 2013; Gill et al., 2014; Shannon et al., 2016). Noise is defined as any environmental condition interfering with acoustic signal transmission and detection (Rabin and Greene, 2002). Under noisy conditions, senders should adapt their acoustic signals to enhance information transfer to the receivers, often reducing the effectiveness of the message (Warren et al., 2006). Adjusting temporal and spectral characteristics of acoustic signals to reduce masking by noise have been reported in several vertebrates (see Warren et al., 2006; Barber et al., 2010). In most anuran species, the acoustic communication plays a critical role in reproduction and adverse effects of auditory masking produced by natural abiotic and biotic sources of noise are known in this group (e.g., Gerhardt and Klump, 1988; Schwartz et al., 2001; Bee and Swanson, 2007; Röhr et al., 2016). Particularly, conspecific choruses produce limiting communication conditions because near acoustic sources simultaneously generate competing calls with a high degree of temporal and spectral overlap and increased noise levels interfering with reproductive behavior (Schwartz et al., 2001; Wollerman and Wiley, 2002; Richardson and Lengagne, 2010). For generations, animal populations have managed to adjust to these natural constraints on communication to improve the effectiveness in transmission and reception of signals. However, anthropogenic noise has created novel environmental pressures that directly affect communication in new ways and could negatively impact animal populations (Rabin and Greene, 2002). The constant increase of urban areas intensifies the emergence of new noisy environments that can have unfavorable effects on animal communication (Sun and

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Narins, 2005; Bee and Swanson, 2007; Cunnington and Fahrig, 2010; Vargas-Salinas et al., 2014). Recent evidence suggests that several anuran species adjust their calls to compensate for increasing levels of anthropogenic noise (Lengagne, 2008; Parris et al., 2009; Cunnington and Fahrig, 2010; Roca et al., 2016; Caorsi et al., 2017). Road traffic noise is one of the most important noise pollution sources causing not only an alteration of signal transmission but also a health threat that could reduce individual survival (Troïanowski et al., 2017).

Several comparisons based on species inhabiting urban or rural habitats were made but it is often difficult to confirm that observed behaviors are specifically due to the effect of noise pollution (Partecke et al., 2006; Troïanowski et al., 2017). It is also possible that call variability are confounded by a response to conspecific chorus sounds rather than a reaction to traffic noise. The aim of our study was to test the prediction that males would change the dominant frequency of their calls out of the noise range and increase the temporal properties of their calls, as proposed by the Acoustic Adaptation Hypothesis (Morton, 1975; Rabin et al., 2003), in areas with traffic and/or conspecific chorus noise. In addition, we compare if the effect is greater in situations of combined noise. For that, we compare calls of the common lesser escuerzo *Odontophrynus americanus* vocally active individuals from different noisy scenarios and identify those call traits varying in each acoustic situation. This species forms large acoustic aggregations and often uses reproduction sites associated with roads, and therefore constitutes an excellent case of study for this research.

2. Material and methods

2.1. Focal species and study area

Populations of *O. americanus* occur in high densities at the study area. Males are small (mean snout–vent length [SVL] = 45.6 mm) and emit pulsed advertisement calls (Grenat et al., 2012, 2017). This species breeds during austral spring-summer months (September–March) and daily reproductive activity takes place mainly between 2000 and 0500 h.

Series of male advertisement calls were recorded in 11 sites from Córdoba and San Luis provinces, Argentina, in the periods 2009–2012 and 2016–2017. We selected temporary and permanent ponds and flooded roadside ditches where individuals of the focal species were known to breed. Five sampling sites near to main entry and exit roads of Río Cuarto city (33°07′23″S − 64°20′52″W) were associated to road traffic noise pollution (TN): S1 (33°00′S − 64°21′W), S2 (32°51′S − 64′21 W) and S3 (32°47′S − 64°20′W) near National Road N° 36, that connects the two main cities of Cordoba province, Río Cuarto and Cordoba; S4 associated to National Road A005 (Río Cuarto bypass road; 33°06′S − 64°22′W); S5 next to Provincial Road N° 30 (33°04′S − 64°28′W). In all cases the annual average daily traffic is greater than 6000 vehicles, reaching 12,500 vehicles in most of the selected sites near to the city (IERAL, 2012; ONDat, 2015). In all sampling sites, road traffic was the most important anthropogenic noise source. The distance from the sampling sites to noise sources was in a range of 10–32.5 m. To characterize the TN sites, noise levels and recording of vehicular traffic were performed. During the hours of greatest activity of local anurans (between 19 and 24 h), measurements of five minutes were made, alternating one minute intervals with and without registration. Noise level was measured from 25 to 30 cm above ground level using a CEM DT-805 sound meter (A-weighted). All TN sites had traffic-noise levels ranging from 45.3 to 79.8 dB(A) L_{10} SPL and the average frequency of noise was 978.13 (± 25,78 Hz). Sampling sites were a minimum of 3 km separated to avoid potential pseudoreplication (Cunnington and Fahrig, 2010). The maximum distance between individual sampling sites was < 40 km.

Additionally, six breeding sites were sampled in pre-mountain and rural areas from center-south Córdoba (S1: 32°42′S − 64°24′W; S2: 32°42′S − 64°28′W; S3: 32°40′S − 64°29′W; S4: 32°40′S − 64°32′W; S5: 32°40′S − 64°37′W) and San Luis (S6: 32°98′S − 64°83′W) and were considered not disturbed by traffic noise (N-TN). These sites were chosen because they are located at least more than 5 km of routes and urban areas. Furthermore, all sites except the sampling site from San Luis (~125 km), were situated relatively near of noisy environments used in the study (~15–45 km), minimizing the potential effect of differences in habitats associated to latitudinal or altitudinal variation.

Most TN and N-TN sites were visited on at least two occasions and individuals in chorus (Ch) and non-chorus (N-Ch) situations were registered. N-Ch condition was considered when < 5 individuals were acoustically actives and advertisement calls were not overlapping.

2.2. Recording and analysis of advertisement calls

Vocal series were recorded from 135 *O. americanus* males (n = 975 calls) using a Walkman Digital AudioTape (DAT) Sony™ TCD-100 with a microphone ECM-MS907 Sony™ and a Tascam™ DR-100 MKII Digital Recorder with an shotgun microphone Rode™ NTG2. Throughout each recording, the microphone was held at around 0.5–1 m from the focal calling male at least for 3 min. Immediately after the acoustic recording, temperature of call site (to the nearest 0.1 °C) was registered and the snout-vent length (SVL) of each individual was measured using a digital calliper (± 0.01 mm).

Acoustic signals of 57 individuals from N-TN sites (N-Ch: n = 21; Ch: n = 36) and 78 individuals from TN sites (N-Ch: n = 21; Ch: n = 57) were digitized and analysed by means of oscillograms, spectrograms and power spectra using the software Adobe® Audition™ 1.0 (sampling rate: 44.1 KHz; bit depth: 16 bit; window function: hamming; frame length: 512 points; grid resolution: 128 points; FFT size: 1024 points; amplitude logarithmic). The call of *O. americanus* consists of a single pulsetrain, with low intensity pulses located at the beginning and the end of call, and pulses increasing in intensity in the middle of call (Fig. 1). Five to ten calls of a single series per individual were characterized by four acoustic properties: (1) call duration (CD) [ms]; (2) dominant frequency (frequency containing the greatest amount of acoustical energy; DF) [Hz]; (3) intercall interval (ICI) [ms] and (4) pulse rate (PR = 1/ (PD + IPI); PD = pulse duration and IPI = interpulse interval). Pulse rate was calculated using at least three measures of PD and IPI in each call.

2.3. Statistical analyses

The arithmetic means of all call parameters were calculated for each series and used for further analyses. Acoustic data were shown to be normally distributed using the Shapiro-Wilk test for normality ($p < 0.05$). Linear regressions between call properties and water temperature were performed to assess the effect of this environmental variable on acoustic traits. Since temperature can affect call properties of anurans (Gerhardt and Huber, 2002), we corrected the acoustical properties significantly correlated with temperature by adjusting their values to 20 °C (mean of registered temperatures) following Heyer and Reid (2003). We also analysed the relationship between SVL and call traits by mean linear regressions, mainly due to the association between body length and spectral variables described in other anuran species (e.g. Gingras et al., 2013). Furthermore, the association between traffic noise level and distance to road of perturbed sites was analysed by mean regression analysis. These analyses were performed using Statgraphic 5.0 for Windows.

We examined variation in call properties using linear mixed models (LMMs), with CD, ICD and DF as response variables and the following fixed predictor variables: Traffic noise (absence/presence), Chorus noise (non-chorus/chorus) and the interaction between them. To control for potential pseudoreplication, sampling site was included as a random effect in all models. A generalized linear mixed model (GLMM) with a binomial error distribution (logit link function) and the same fixed and random factors was used for non-normally distributed

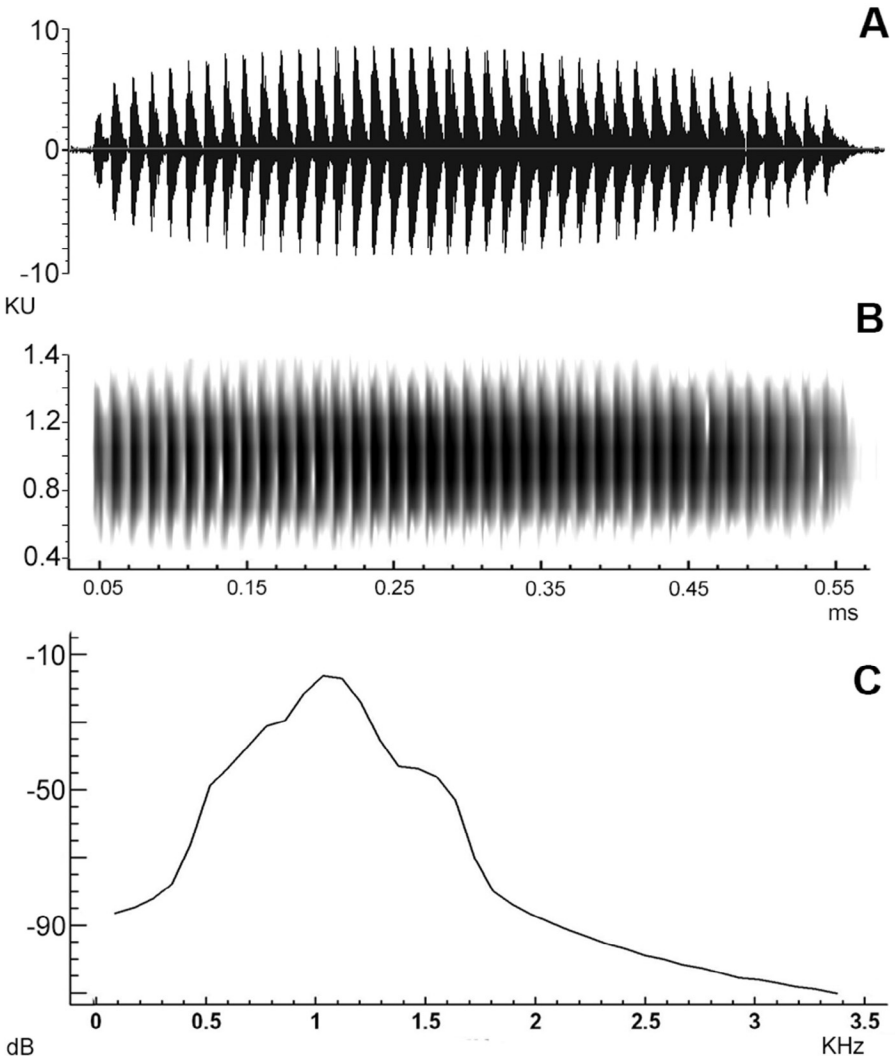

Fig. 1. Oscillogram (A), spectrogram (B) and power spectrum (C) of a representative advertisement call of *Odontophrynus americanus*. SVL: 44.4 mm; temperature: 18.1 °C; site S2 (N-TN).

parameter, PR. We used the Akaike's Information Criterion (AIC) to determine the model that better fit the data. For significant effect interactions, post hoc tests (Fisher LSD) were Bonferroni-corrected to examine pairwise differences between noise conditions.

Multivariate approach based on Principal Component Analysis (PCA) was performed to identify the acoustical properties that best accounted for variation among all possible noise conditions (N-TN/N-Ch; N-TN/Ch; TN/N-Ch and TN/Ch). LMMs and PCA were conducted using InfoStat (Di Rienzo et al., 2012) and R 3.3.2 (R Core Team, 2016).

3. Results

Temperature was positively associated with PR ($r = 0.53$; $p < 0.0001$) and negatively with CD ($r = -0.39$; $p < 0.0001$) and ICI ($r = -0.23$; $p < 0.05$). Temperature was not correlated with DF ($r = -0.098$; $p = 0.2467$). Table 1 shows means and standard deviations for temperature-adjusted (20 °C) acoustic properties of advertisement calls of individuals from TN and N-TN sites in chorus and non-chorus conditions.

The regression analysis showed a non-significant relation between the spectral parameter (DF) and SVL ($r = -0.1874$; $p = 0.4038$). Furthermore, there was no relation between male SVL and any temporal property (CD: $r = -0.043$, $p = 0.8495$; PR: $r = -0.115$, $p = 0.6099$; ICI: $r = 0.015$, $p = 0.9522$). There was no significant differences in SVL between individuals from sites with and without traffic noise (ANOVA, F = 2.06; p = 0.1695).

No significant association was detected between traffic noise level and distance to road of perturbed sites ($r = -0.1068$; $p = 0.1760$). LMMs and GLMM (Table 2) showed that traffic noise had a statistically significant effect on PR and DF with higher values in TN than N-TN sites ($p < 0.01$, Fisher's LSD test) while a significant increase in ICI and a decrease in DF ($p < 0.01$, Fisher's LSD test) was found in presence of chorus noise (Table 2). Traffic noise*chorus noise interaction had a significant effect on DF, PR and ICI. Fig. 2 shows that individuals significantly increased the DF in presence of noise being the highest values found in sites with combined effect of vehicular traffic and chorus noise (TN/Ch; $p < 0.01$, Fisher's LSD test). PR showed no differences between TN and N-TN in absence of chorus while in chorus situations, PR increased in N-TN sites and decreased at sites associated to roads (TN). ICI was significantly higher in sites without traffic and chorus noise (Fig. 2; $p < 0.01$, Fisher's LSD test). CD showed no differences between traffic or chorus noise effects or for the interaction between them (Table 2).

The first two principal components (PC1 and PC2) explained 93.1% of the total variation. The relative contributions of the different acoustic parameters to PC1 and PC2 are illustrated in the PCA biplot (Fig. 3). PC1 (63.1%) clearly separated males calling in noisy conditions from males vocalizing in sites without traffic and chorus noise and was

Table 1
Mean and standard deviation of acoustic measurements taken on *Odontophrynus americanus* call series from sites with (TN) and without traffic noise (N-TN) in chorus and non-chorus conditions. Parameters abbreviations: CD: call duration; DF: dominant frequency; PR: pulse rate; ICI: intercall interval. N: number of calls analysed.

Sites	N	Acoustical parameter			
		CD (ms)	ICI (ms)	PR	DF (Hz)
N-RTN					
Non-Chorus	178	479.1 ± 40.7	2012.1 ± 689.6	84.3 ± 3.9	906.13 ± 53.6
Chorus	252	490.6 ± 52.4	1354.7 ± 621.1	91.2 ± 10.2	1023.82 ± 49.1
RTN					
Non-Chorus	152	498.2 ± 49.7	1545.1 ± 365.6	83.1 ± 7.7	1001.1 ± 75.1
Chorus	393	492.9 ± 74.2	1611.4 ± 919.2	78.7 ± 7.1	1074.6 ± 72.7

associated mainly to higher DF (− 0.58) and CD (− 0 5 7). PC2 (30.8%) had high positive loading for PR (0.90) and separated males from N-TN and TN sites calling under chorus situation.

4. Discussion

The impact of anthropogenic and conspecific chorus noise on call properties of *O. americanus* was studied in the species' habitats. Our results demonstrate that males modify their calls in noisy situations and that there is a differential variation of acoustical properties depending on the background noise type to which they are subject. Furthermore, the combination of traffic and conspecific chorus noise seems to have a greater effect on calls of individuals, potentially involving an adjustment of acoustic properties toward more extreme values. These results highlight the importance of conducting *in situ* behavioral studies taking into account the additive effects produced by different sources of environmental noise instead of analyzing each separately.

4.1. Effect of biotic and anthropogenic noise on call spectral and temporal properties

Several experimental and observational studies have reported frequency shift in birds, insects and anurans exposed to noise to avoid auditory masking and minimizing the signal attenuation (e.g., Parris et al., 2009; Lampe et al., 2014; Roca et al., 2016). In agreement with these studies, we found significant differences in the DF of callers from acoustically disturbed environments. In addition, in PCA, this property showed a strong association with the component separating groups of males calling in noise situations. One possible confounding variable explaining the frequency variation could be the effect of body size on the dominant frequency of calls. Spectral properties of most anuran advertisement calls are directly related to the frequency at which the vocal cords vibrate, and therefore depend upon their mass and tension, strongly associated with body size (Castellano and Giacoma, 1998). However, we do not find a relation between this acoustic property and SVL and no differences were observed between SVL of males from acoustically disturbed and undisturbed sites, so we assume that the variation observed is related to noise exposure.

The response of males in presence of conspecific choirs and/or road traffic noise was always to increase the DF of their calls. In some vocal animals such as birds it is expected that species producing typical call frequencies that overlap with the background noise to show positive changes in this property, which is predicted by the Acoustic Adaptation Hypothesis (Rabin et al., 2003; Goodwin and Shriver, 2011). However, shifting call frequencies is not a general rule in anurans (Roca et al., 2016). While some anuran species increase their dominant frequency in noisy scenarios (*Litoria ewingii* – Parris et al., 2009; *Rana clamitans* and *R. pipiens* – Cunnington and Fahrig, 2010) other species shift negatively or did not show any shift in this property (*Hyla arborea* – Lengagne, 2008; *Crinia signifera* – Parris et al., 2009; *H. versicolor* and *Bufo americanus* – Cunnington and Fahrig, 2010; *Pseudacris crucifer* – Hanna et al., 2014). These differences could be related to the greater or lesser overlap of the peaks of call frequency of the different species with the relatively low frequencies of traffic noise (< 2 KHz).

Because anurans do not have learned calls, significant frequency shifts could take many generations to manifest at population levels (Parris et al., 2009). The long-term exposition for multiple generations to naturally noisy background, such as conspecific chorus, could have improved the mechanisms to decrease interferences between acoustic signals and environmental noise (Lengagne, 2008). However, conspecific chorus and anthropogenic noises differ in their spectral structure. While a conspecific chorus has a narrow frequency range, anthropogenic noise is variable and composed of low frequencies mainly below of 2000 Hz. Particularly, the traffic noise had its main energy below 1000 Hz (Warren et al., 2006; Nemeth and Brumm, 2010; our study). Previous studies showed that *O. americanus* has an average DF ranging between 850 and 1050 Hz (Martino and Sinsch, 2002; Grenat et al., 2017) so that anthropic noise could affect it directly. When acoustic signals and background noise overlap in frequency, auditory masking may occur (Brumm and Slabbekoorn, 2005), and in this species this is maximized in chorus situations. Modifications in detection thresholds and a reduction in the discrimination capacity between signals by the receivers are direct effects of auditory masking (Wiley, 1994; Bee and Swanson, 2007). In comparison with individuals vocalizing at acoustically undisturbed sites, our results show that individuals exposed to noise scenarios increased their DF in average

Table 2
Results from the best LMM and GLMM fit for the variables analyzed. Models include Traffic noise (TN), Chorus noise (Ch) and TN*Ch as fixed factors and sampling site as random factor. AIC = Akaike Information Criterion. P-values significant at the 0.05 level are in bold. CD = call duration; ICI = intercall interval; PR = pulse rate; DF = dominant frequency.

Factor	CD LMM AIC = 1411.01		ICI LMM AIC = 2031.72		PR GLMM AIC = 887.78		DF LMM AIC = 1431.88	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
Traffic Noise (TN)	1.13	0.2727	0.76	0.3835	25.88	< **0.0001**	34.49	< **0.0001**
Chorus (Ch)	0.18	0.6734	6.04	**0.0153**	0.67	0.4159	70.78	< **0.0001**
TN * Ch	0.78	0.3925	9.06	**0.0032**	16.95	**0.0001**	4.39	< **0.0398**

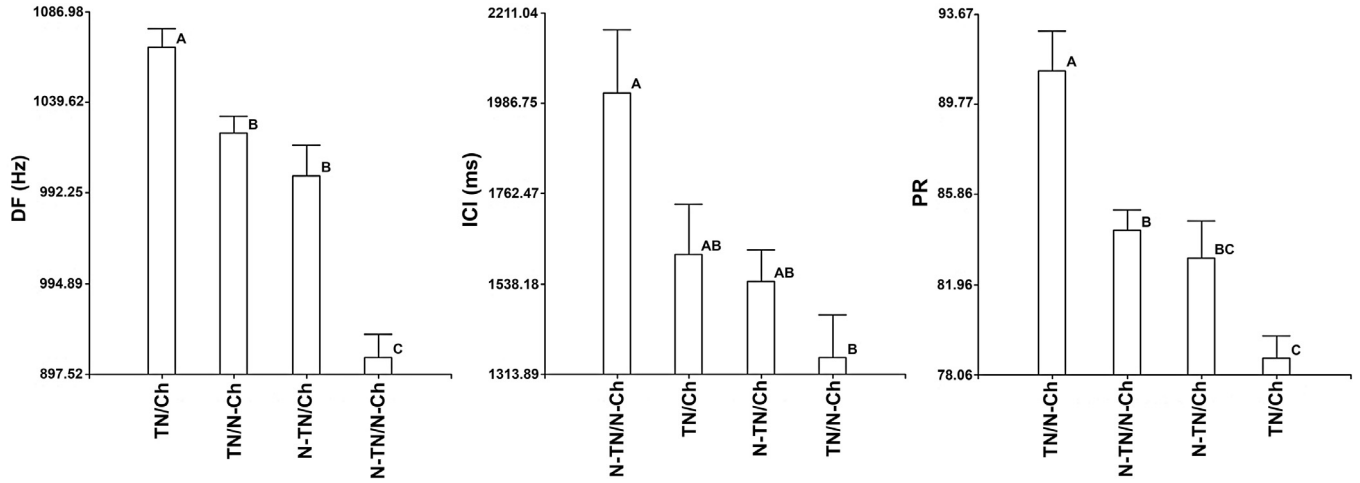

Fig. 2. Comparison of mean values (± SE) of call variables of males calling in all possible noise conditions: without traffic and chorus noise (N-Tn/N-Ch); with traffic and chorus noise (TN/Ch); with traffic noise in absence of chorus (TN/N-Ch); and without traffic noise in presence of chorus (N-TN/Ch). Only the call variables whose interaction between fixed effects was significant (LMM and GLMM; $p < 0.0001$) are shown. Different letters represent significant differences between means ($P < 0.01$) (Fisher's LSD test). DF = dominant frequency; PR = pulse rate; ICI = intercall interval.

about 13%, in presence of chorus noise and 20% (with maximum frequency peaks exceeding 30%) under a combination of both anthropogenic and biotic noise. Changes in this proportion in call frequency have been reported in other anuran species in noisy environments (e.g. *Litoria ewingii*, Parris et al., 2009; *Rana clamitans* and *Rana pipiens*, Cunnington and Fahrig, 2010). While calling at high frequencies could reduce signal efficiency because higher frequencies attenuate more rapidly in relation to lower frequencies (Bee et al., 2001), males calling with DF above background noise could be more easily distinguished and chosen by the females. Although playback experiments have demonstrated that frequency shifts of this proportion are detectable by females of some anuran species in absence of masking noise, it has not been field-tested (Gerhardt, 1991; Wollerman and Wiley, 2002; Parris et al., 2009).

In addition to observed frequency shifts, call temporal traits of *O. americanus* showed a differential variation under noisy environments. Analyzing the temporal properties, we observed that although CD remained unchanged, *O. americanus* males decreased the ICD in presence of chorus or traffic noise. Nevertheless, when both effects were combined, males increased their ICDs. This parameter is a measure of

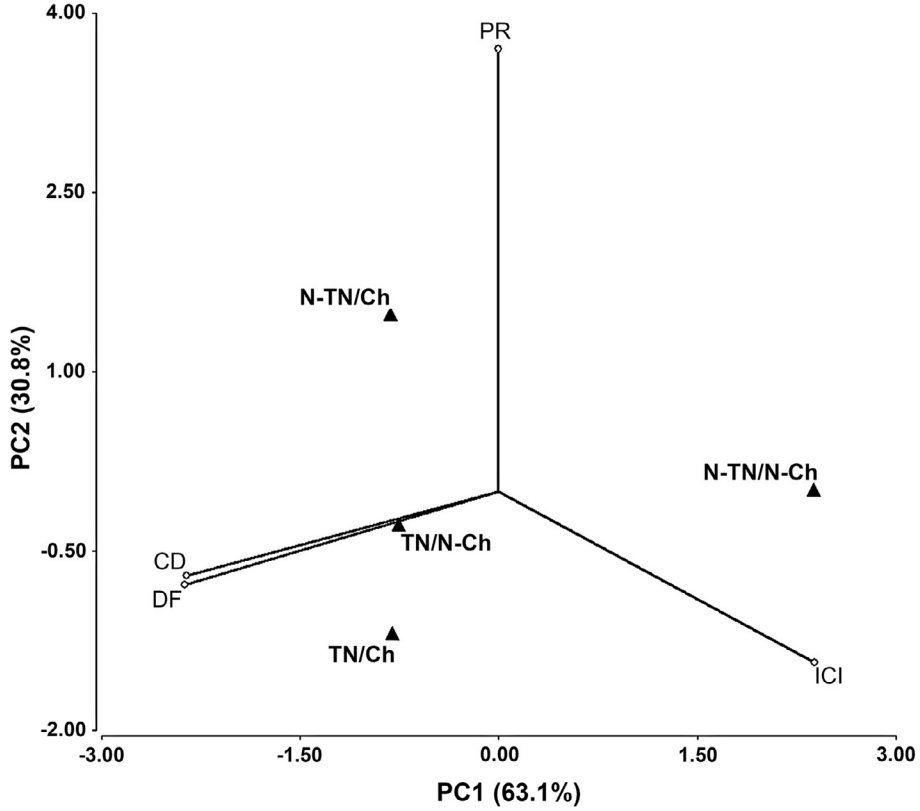

Fig. 3. Biplot (PCA) based on acoustical parameters in four noisy conditions: without traffic and chorus noise (N-Tn/N-Ch); with traffic and chorus noise (TN/Ch); with traffic noise in absence of chorus (TN/N-Ch); and without traffic noise in presence of chorus (N-TN/Ch).

calling rate in which greater ICD result in slower call rates and vice-versa (Pfennig and Ryan, 2007). Anuran females mostly choose faster call rates to slower rates (e.g., Cherry, 1993; Wagner and Sullivan, 1995). Because calling faster involves more energy, call rate is considered a condition-dependent trait, revealing reliable information about male quality (Cherry, 1993). Although some studies suggest that amphibians increase their call rate for improving call detectability in noisy conditions (Penna et al., 2005; Kaiser and Hammers, 2009), males of several species tend to decrease their vocal activity, probably to avoid degradation or masking of significant call traits (e.g., Vargas-Salinas et al., 2014). Furthermore, it is known in other vocal animals that increasing the frequency, as has been observed in our study, can make it difficult to call at a high rate (Morton, 1975; Wiley and Richards, 1982).

In the analysis of temporal fine structure, we observed a reverse effect of chorus situations on PR of males from N-TN compared to those calling in TN conditions. Callers from N-TN sites increase the PR in presence of chorus, which could be considered a normal response of the species in this noise situation (Schwartz et al., 2001; Penna et al., 2017). However, PR values found in TN/Ch condition show that the male response could be completely opposite in scenarios with traffic noise. Grenat et al. (2017) observed that PR showed the lowest ratio between-within individual variation suggesting that this property have been subject to stabilizing selection and could be involved primarily in species recognition in *O. americanus*. In many anuran species, species-specific narrow ranges of values of PR are used to identify conspecific mates (e.g., Gerhardt, 1991; Castellano and Giacoma, 1998; Schul and Bush, 2002). Under a combined effect of conspecific chorus and traffic noise, individuals have low PR values, diverging from the average values reported for the species (Grenat et al., 2017). Therefore, if the changes in these traits are immediate or short-term, females could fail not only to detect the quality of males, but also in the conspecific identification. Female choice tests would be necessary to test if these changes in the call properties significantly affect the detection and discrimination of conspecific males, mainly in noisy field environments.

4.2. Implications for the ecology, evolution and conservation of anurans inhabiting noisy environments

The common lesser escuerzo *O. americanus*, as several other anuran species, are often found reproducing in very noisy environments suggesting that these species do not avoid acoustical disturbed areas to breed. Although the *O. americanus* males showed variations in both spectral and temporal call properties, many species may not be effective enough by adjusting their acoustic signals in the short term to manage the attenuation, distortion and/or interference produced by noise, even within the same group of organisms. For example, males of *H. arborea* exposed to high noise levels do not seem capable to modify the call frequency or duration to improve the signal transmission (Lengagne, 2008). In this sense, some adaptive acoustic responses could be physiologically impossible (Katti and Warren, 2004). These variations in acoustic adaptability could explain, at least partially, why some species are more successful in noisy environments and others are not (Barber et al., 2010).

For anurans males, calling is potentially the most energetically costly activity (Pough et al., 1992) and the positive directional response in DF of *O. americanus* under both different levels and types of noise supposes a greater energetic cost. For this, such changes are very difficult to maintain over long periods (Parris et al., 2009). However, this cost is considerably less than that of increasing the calling rate (Parris, 2002). Thus, the decrease in call rate observed in *O. americanus* and other species exposed to road traffic (Cunnington and Fahrig, 2010) could energetically compensate for the increase in frequency, and could represent a better strategy of callers in very noisy environments, in which silence intervals are limited. However, if these adjustments produce direct or indirect modification on call properties related to species recognition (PR) acoustic divergence between conspecific populations among habitats consistently differing in noise conditions could occur (Slabbekoorn, 2004).

Although several authors have reported changes in acoustic signals, it is unclear whether the adjustments in call properties compensate for potential effects of noise disturbance on breeding and if the effect is differential under different levels or types of noise. Breeding success data of anurans from noisy areas is necessary to determine whether noise pollution significantly affects amphibian populations and reduce the individual reproductive success.

Finally, since acoustic communication is crucial to the reproductive success of most anurans and in the face of increasing global concern about the anuran decline, the understanding of how these animals respond to noise is very important. Despite this, bioacoustics is still scarcely used in conservation studies on anurans compared to their use in other vocalizing animals, particularly in birds (e.g. Slabbekoorn and Ripmeester, 2008). Such behavioral studies can make important contributions to species conservation and generate key information that can be used in management plans, benefiting not only anurans but also other animal groups that use these habitats. It is important to note that often the extrapolation of results to other species may be inappropriate because each species can respond differently to a disturbed environment. Through an experimental approach using noise playback, Lengagne (2008) observed that males of *H. arborea* vocalizing in a chorus situation were less affected by traffic noise pollution. Based on our results, males of *O. americanus* showed strong adjustments in call properties when both traffic noise and conspecific chorus are present, although an experimental approach would be necessary to reinforce this result. In any case, these results show that it is relevant to study and take into account the social context for a better prediction of noise effects on anuran populations.

In the last time, several developing countries consider noise pollution data in environmental impact studies and to implement different mitigation measures for road and urban noise (Forman et al., 2003). For example, vegetation and solid sound barriers along roadsides are suggested to reduce the noise effects (Parris et al., 2009; Maleki and Hosseini, 2011). On the other hand, although more difficult in less developed countries, landscape-level planning process to minimize future habitat fragmentation the impact on relevant natural areas would be necessary (Beebee, 2013), especially in areas where threatened anuran species reproduce. In this way, a reduction of anthropic noise would allow species to deal mainly with natural noises, for which they have developed different strategies throughout the generations improving the effectiveness of their signals. However, we agree with Beebee (2013) that to mitigate the effects of roads on wildlife, a modification in human behavior is necessary and urgent.

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