

Animal Bioacoustics: Paper ICA2016-866**Development of a computational tool to analyze sounds: A biological study with anuran**

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Abstract

The emission of underwater sounds in anuran tadpoles has been documented in only two species from Argentina and one from Madagascar. Underwater sound emission by *Ceratophrys ornata* (Anura: Ceratophryidae) tadpoles was a novel finding reporting the first evidence in anuran larvae. The sound has been described as part of an antipredator mechanism that diminishes the frequency of predation between conspecifics. The aim of the study was to describe sound variability from tadpoles to adults with a novel technique in bioacoustics. Sounds emitted by tadpoles were both recorded underwater and out of water. The recording system consisted of a microphone and an interface coupled with a laptop. It was first calibrated with an acoustic reference source and compensated in order to obtain a recording system with flat frequency response. Audio recordings were digitalized, and post processed by a computational tool specifically developed in a numerical environment software. Variables selected to describe basic structure of sounds were: sound duration, number of pulses, number of inter pulses and dominant frequency. These variables were supplemented with typical acoustic parameters, such as equivalent continuous pressure level, peak sound pressure level, and spectral analysis with constant bandwidth filters. The interdisciplinary experience allowed developing a reliable system of recording, analysing thousand of sounds in a short period and therefore characterizing the sound of a species considering all variability.

Keywords: bioacoustics, sound analysis, anuran larvae

Development of a computational tool to analyze sounds: A biological study with anuran

1 Introduction

Most adult anurans emit sounds in the human audible range of frequencies (from 20 Hz to 20 kHz; [1]) and others into the ultrasonic range (above 20 kHz; [2]). Adult anurans produce diverse types of acoustic signals, the best known being advertisement calls that are produced by sexually active males and distress calls that are produced by both sexes after disturbances [1]. Both calls have been thoroughly described for many species of anurans since century XX. Unlike the degree of progress achieved in the bioacoustics of adult anurans, research on the emission of sounds by their larvae is scarce. The first publication that documented the emission of underwater sounds by anuran larvae involved *Ceratophrys ornata* [3]. Such finding had great impact in the scientific field and was considered important for various biological disciplines such as Ecology, Ethology and Bioacoustics. In this sense, a doctoral thesis describing the sounds of this species from tadpole to adult was developed [4]. Nowadays, only two species of anuran larvae had been described to produce sounds: the aforementioned species belonging to the Ceratophryidae family [3] and another species belonging to the Mantellidae family, *Gephyromantis azzurrae* [5]. In both cases, sounds were associated with their macrophagic and carnivorous habit.

In the field of Bioacoustics, sound recordings are usually done with a tape recorder or a digital recorder connected to a directional microphone. Nevertheless, it is not a frequent practice to work with calibrated instruments as in the acoustic field. Moreover, analysis of sounds require using specific programs to visualize the oscillogram (sound duration / amplitude) and spectrogram (sound duration / frequency). However, those programs do not have a proper routine to automatically analyze each sound. Herein, the analysis of lots of sounds by manual procedures take plenty of time. Considering these issues, the aim of the present work was to reduce time by post-processing hundreds of sounds with a computational tool specifically developed in a numerical environment software.

2 Work method

2.1 Breeding and maintenance of the study species

Selected species, *Ceratophrys ornata*, belongs to the family Ceratophryidae [6], is known as “Ornate Horned Frog” and is distributed in the Pampas region of Argentina and Southern Brazil. It is a sit-and-wait predator species with a robust and colourful appearance and a carnivorous habit from larvae to adult [7, 8].

Adults of the selected species were collected in La Plata city and maintained in the laboratory in a 34-L terrarium containing soil and water, simulating their natural habitat, at 25 ± 1 °C and with a photoperiod of 16:8 light: dark. Egg laying was induced by injecting pairs of adults intraperitoneally with 0.4 µg/g des-Gly10, D-Ala6, Pro-NHEt9-GnRH, plus 10 µg/g

metoclopramide hydrochloride dissolved in 0.7% NaCl according to the AMPHIPLEX method [9]. Pairs laid an average of 3370 ± 1568 fertile eggs after 12 h of injection. Eggs and tadpoles were reared under the same conditions as adults but using dechlorinated tap water (temperature 22 ± 1 °C, pH 7.92 ± 0.15 , hardness 122.71 ± 21.26 mg CaCO₃/L) with continuous aeration until they reached the desired developmental stages of Gosner [10].

2.2 Recording sound

The emission of sounds was evaluated from stage 25 till metamorphosis (stage 46). Sounds were recorded with a directional microphone CADe70 (CAD Audio, Solon, USA), with a cardioid capsule, covered with a latex sheet to protect it from water, at a distance of 15 cm from larvae. The microphone was connected to a laptop by a I/O interface GuitarRig Session (Native Instruments, USA). The recording chain was first calibrated inside an acoustic room using a sound level meter B&K type 2250 (Brüel and Kjær Sound and Vibration Measurement A/S, Denmark). The linearity of all the recording chain was compensated to obtain a flat response at all frequencies tested.

All sounds were recorded under controlled laboratory conditions, in test chambers with high acoustic isolation from their environment and low reverberation time. However, since sounds have very low pressure levels, post-processing procedures were implemented to reduce the noise [11].

Moreover, in order to obtain calibrated parameters it is essential to have a calibration signal. Such a signal is generated by an acoustic source reference, recorded with the same system, and registered in a WAV file. In this case, the acoustic reference was a B&K type 4231 (94 dB / 114 dB @ 1kHz).

2.3 Signal processing and computational tool

Recordings were stored in WAV format with a sampling rate of 44,100 Hz. Selected sounds ($n = 100$) were cut from the original file so that each contained a single sound. Since times of silence at the beginning and end of the recordings were minimal, this processing was linked to the calculation of sound duration.

Sound recordings were then stored in the bank files for further processing by applying a mathematical computational tool designed in an environment 'ad hoc', in order to calculate its characteristic acoustic parameters.

Bioacoustic variables selected to describe basic structure of sounds were: sound duration (in milliseconds), number of pulses, number of interpulses and dominant frequency (in hertz). These bioacoustic variables were supplemented with typical acoustic parameters, such as equivalent continuous pressure level, peak sound pressure level, and spectral analysis with constant bandwidth filters.

The functioning of the designed software has been schematized in a block diagram (Figure 1). The program was set to take each selected file from the database and condition the signal by using a passband filter in order to eliminate low and high frequency noise that does not belong

to the sound itself but is residual noise. This procedure improved the signal / noise ratio and thus provided a more accurate analysis.

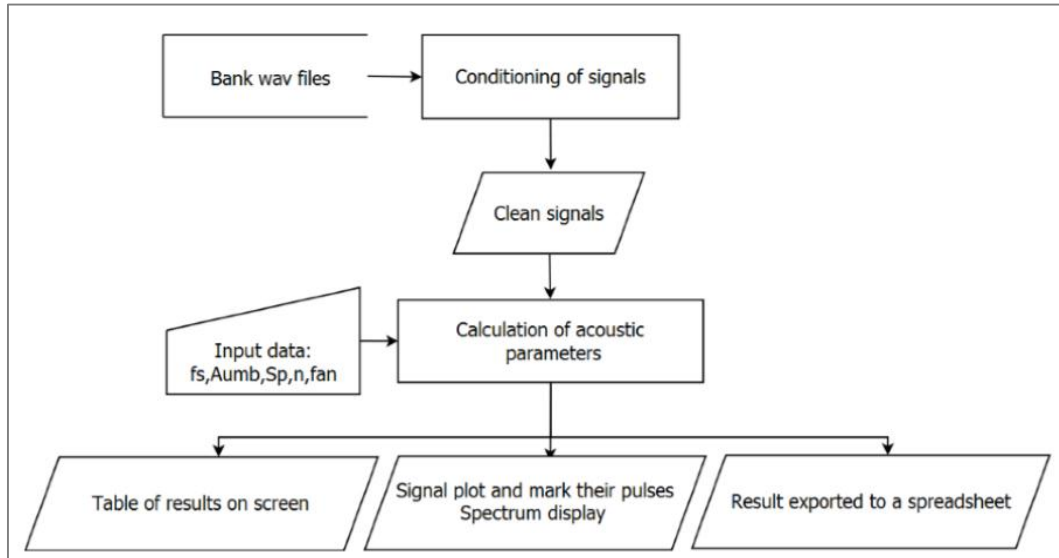


Figure 1: Block diagram of the designed software. fs: sampling frequency in hertz (Hz); Aumb: threshold amplitude of a pulse in pascals (Pa); Sp: minimum separation between pulses in milliseconds (ms); n: number of dominant frequency to calculate; fan: frequency range under analysis in hertz (Hz)

The software also allowed to determine the following acoustic parameters:

2.3.1 Sound duration (S_d , in ms):

The duration of each sound was obtained from the duration of the trimmed WAV file and the frequency sampling (equation 1).

$$S_d = \frac{\text{Length (signal)}}{f_s} \quad [ms] \quad (1)$$

Where:

Length (signal) : length of sampling vector of the signal
 f_s : sampling frequency

2.3.2 Determination of the number of pulses (N_p):

In order to detect and count the number of pulses, the program was set to search the local maxima of the signal, by applying restrictions on amplitude and location relative to adjacent peaks. The acoustic parameters chose to recognize a pulse within the structure of a sound were: the minimum separation between pulses (Sp), and the threshold amplitude ($Aumb$, minimum amplitude considered as a possible pulse) (Figure 2). Therefore, the software recognized a pulse as the highest local maximum within the time interval given by the minimum separation of pulses.

2.3.3 Determination of the number of interpulses (Nip):

Once determined the number of pulses of each sound, the number of interpulses was obtained by decreasing the number of pulses in one.

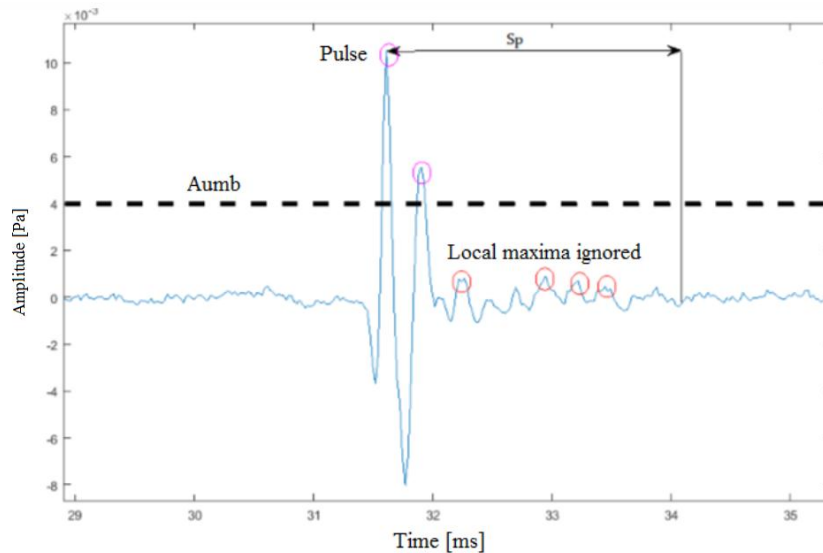


Figure 2: Determination of the number of pulses by the developed software. Aumb: threshold amplitude of a pulse in pascals (Pa); Sp: minimum separation between pulses in milliseconds (ms); Time in milliseconds (ms); Amplitude in pascals (Pa)

2.3.4 Determination of the dominant frequency (Df, en Hz):

In order to obtain the dominant frequency a spectrogram was performed. The ordinary way of presenting the results was by a three-dimensional graphic: time in X axis, frequency in Y axis, and amplitude using a colour scale (Figure 3).

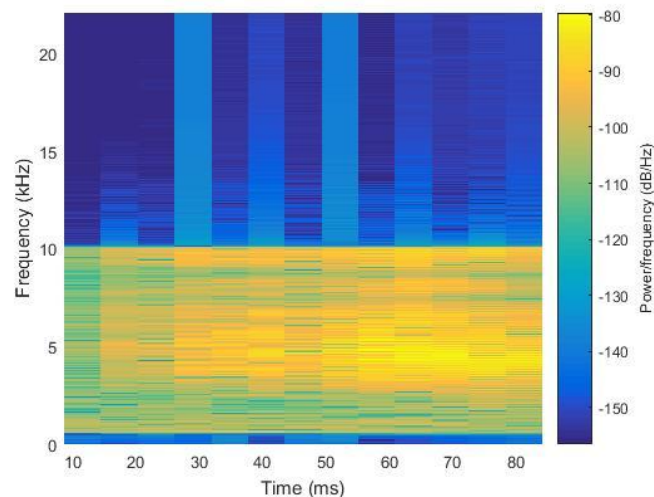


Figure 3: Spectrogram obtained by FFT filters (BW = 43 Hz) of the sound emitted by a single larva at stage 37 of development. ms: milliseconds; kHz: kilohertz; dB: decibels

Time evolution of the spectrum was represented by applying a filter of constant bandwidth (FFT, Fast Fourier Transform) to the sound. A matrix with sound levels of the signal was generated from the output vector of each filter at regular intervals of time. Hence, the frequency associated with the higher level component was extracted. This procedure could be done rapidly and efficiently by the numerical calculation software.

2.3.5 Determination of the equivalent continuous sound level (L_{eq} , in dB):

The equivalent continuous sound level is an energy-time average of the signal obtained from a linear integration. For continuous signals, this parameter is defined in IEC 61672 Standard [12], and for discrete signals [13], the corresponding expression is the equation (2):

$$L_{eq,T} = 20 \log \left\{ \frac{X_{sig}}{P_0} \right\} = 20 \log \left\{ \left[\frac{1}{N_{sig} T_s} \sum_{n=1}^{N_{sig}} p^2(n) T_s \right]^{1/2} / P_0 \right\} \quad [dB] \quad (2)$$

Where:

- X_{sig} : is the RMS value of the sound pressure digitalized signal
- $p(n)$: is the instantaneous sound pressure vector of the digitalized signal
- N_{sig} : is the number of samples of the sound pressure vector of the digitalized signal
- T_s : is the sampling period (1/44,100 Hz)
- P_0 : is the reference pressure (20 μ Pa)

In order to obtain a calibrated sound level a calibration signal was used, being the calculation process as presented in equation (3):

$$L_{eq,T} = 20 \log \left(\frac{X_{sig}}{X_{cal}} \right) + L_{cal} \quad [dB] \quad (3)$$

Where:

- X_{sig} : is the RMS value of the sound pressure digitalized signal
- X_{cal} : is the RMS value of the calibration signal
- L_{cal} : is the sound pressure level of the calibration signal (94 dB @ 1 kHz)

2.3.6 Determination of the peak sound pressure level (L_{PEAK} , in dB):

For continuous signals, the IEC 61672 [12] standard defines the peak sound pressure level as twenty times the logarithm to the base ten of the ratio of a peak sound pressure to the reference sound pressure. For discrete signals the peak sound pressure is defined in equation (4).

$$P_{peak} = \text{Max}(\text{signal}) \quad [Pa] \quad (4)$$

Where:

- $\text{Max}(\text{signal})$: maximum of sampling vector of the signal

To obtain a calibrated peak sound pressure level, a calibration signal was used, being the calculation process as presented in equation (5).

$$L_{PEAK} = 20 \log \left(\frac{P_{peak}}{X_{cal}} \right) + L_{cal} \quad [dB] \quad (5)$$

Where:

P_{PEAK} : is the peak sound pressure in pascals

X_{cal} : is the RMS value of the calibration signal

L_{cal} : is the sound pressure level of the calibration signal (94 dB @ 1 kHz)

2.3.7 Spectral analysis with constant bandwidth filter (FFT):

The signals were processed with constant bandwidth filters of 10.8 Hz (by an algorithm that applies FFT), and presented in a two-dimensional graph (frequency in X axis vs. sound pressure in Y axis).

This analysis differed from the spectrogram since it did not show the temporal evolution of the spectrum.

3 Results

The program outputs allowed to obtain the following data:

- Display: A table with data of calculated parameters. As an example, in Table 1 is shown the data obtained with the developed computational tool for 10 recordings, corresponding each to a sound emitted out of water by a different larva at stage 31.
- Display: A list of analyzed signals from which the user can choose a graphical display of the waveform indicating their pulses (see an example in Figure 5), or a graphical display FFT spectral analysis (see examples in Figure 6).
- The possibility to export results to a spreadsheet.

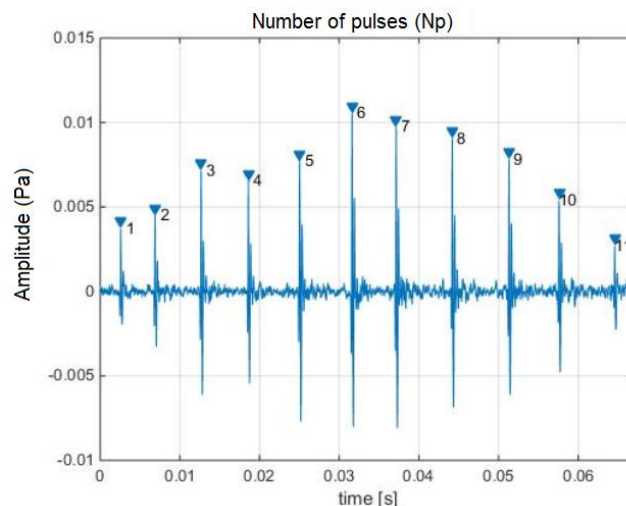


Figure 5: Waveform corresponding to an analyzed signal indicating the number of pulses (Np); Time in milliseconds (ms); Amplitude in pascals (Pa)

Table 1: Calculated parameters from sounds (s) emitted by *Ceratophrys ornata* larvae at stage 31. Sd: sound duration in milliseconds (ms), Np: number of pulses; Nip: number of interpulses; Df: dominant frequency in hertz (Hz); Equivalent sound pressure level in decibels (dB); Peak sound pressure level in decibels (dB)

Sounds	Sd [ms]	Np	Nip	Df [Hz]	Leq [dB]	Lpeak [dB]
s1	56	13	12	3790	39.4	56.2
s2	61	14	13	3059	44.9	64.2
s3	90	14	13	3382	41.4	63.0
s4	66	11	10	3342	45.2	65.0
s5	54	14	13	5082	46.9	63.9
s6	46	13	12	4911	48.8	66.5
s7	69	18	17	4307	49.4	68.2
s8	51	15	14	4287	49.8	68.8
s9	54	11	10	5202	48.5	67.9
s10	64	18	17	4565	54.4	69.9

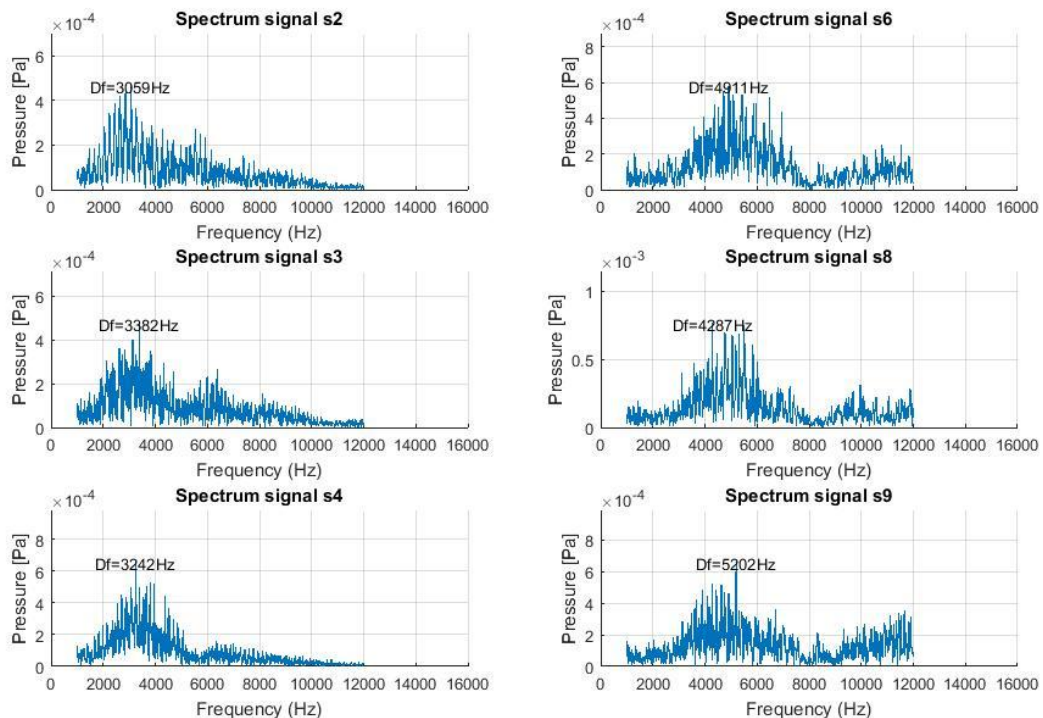


Figure 6: Spectral analysis obtained by FFT filters (BW = 10.8 Hz) of sounds (s) emitted by larvae at stage 31 of development. Frequency in hertz (Hz); Pressure in pascals (Pa); Df: dominant frequency in hertz (Hz)

4 Validation of results

In order to evaluate the program, 50 sounds emitted by different tadpoles on different developmental stages were selected. They were analyzed both manually and with the computational tool. Then, a paired t test was performed for each bioacoustic variable (Sd, Np, Nip and Df). According to these statistical analysis there are no significant differences between variables analyzed by both methods (Sd: $p = 0,99$; Np/Nip: $p = 0,20$; Df: $p = 0,11$). Moreover, in order to numerically determine the intensity of the linear relationship between bioacoustic variables analyzed by both methods, the determination coefficient was calculated. Statistical analysis indicate that Sd ($r^2 = 0.99$) and Df ($r^2 = 0.75$) are more related than Np or Nip ($r^2 = 0.35$).

5 Conclusions

During the development of Salgado Costa's thesis [4] sounds were analyzed manually with complementary software. Since manual procedure takes too much time to analyze lots of sounds, only 20% of the recorded sounds could be included in the thesis. Now, the developed computational tool will allow analysing with reliance hundreds and thousands of sounds in a short period. Nevertheless, the way of measuring Np/Nip must be improve so as to obtain a better linear relationship between variables analyzed both manually and with the computational software. Acoustic parameters allowed supplementing the description and obtaining a global characterization of each sound which can be calculated from the recorded signal processing. Moreover, graphical tools contributed to the study and detection of patterns and anomalies in different signals under analysis.

Moreover, the interdisciplinary experience allowed developing a reliable system of recording, analysing thousands of sounds in a short period and therefore characterizing the sound of a species considering all variability.

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References

- [1] Duellman, W.E. and Trueb, L. *Biology of amphibians*, JHU Press, London (England), 1994.
- [2] Feng, A.S., *et al.* Ultrasonic communication in frogs. *Nature*, Vol. 440 (7082), 2006, pp 333-336.
- [3] Natale, G.S., *et al.* Underwater acoustic communication in the macrophagic carnivorous larvae of *Ceratophrys ornata* (Anura: Ceratophryidae). *Acta Zoologica*, Vol. 92 (1), 2011, pp 46-53.

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- [4] Salgado Costa, C. Desarrollo de un modelo experimental con larvas de *Ceratophrys* spp. (Anura: Ceratophryidae) para su aplicación en estudios ecotoxicológicos de plaguicidas: efectos sobre variables convencionales y bioacústicas. Tesis de la Facultad de Ciencias Naturales y Museo, Universidad Nacional de La Plata, 2016.
- [5] Reeve, E., et al. Acoustic underwater signals with a probable function during competitive feeding in a tadpole. *Naturwissenschaften*, Vol. 98 (2), 2011, pp 135-143.
- [6] Frost, D.R., *Amphibian species of the world: an online reference*. American Museum of Natural History, New York, USA, Version 6.0., 2016.
- [7] Cei, J.M., *Amphibians of Argentina*, *Monitore Zoologico Italiano*, Firenze (Italia), Vol. 2, 1980.
- [8] Gallardo, J.M., *Anfibios de los alrededores de Buenos Aires*. Editorial Universitaria de Buenos Aires, Buenos Aires (Argentina), 1974.
- [9] Trudeau, V.L., et al. Hormonal induction of spawning in 4 species of frogs by coinjection with a gonadotropin-releasing hormone agonist and a dopamine antagonist. *Reproductive Biology and Endocrinology*, Vol. 8 (1), 2010, p 36.
- [10] Gosner, K.L. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica*, Vol. 16 (3), 1960, pp 183-190.
- [11] Vechiatti, N., et al. Bioacústica de anuros: medición en cámara anecoica de sonidos emitidos por larvas. XIII Congreso Argentino de Acústica. VII Jornadas de Acústica, Electroacústica y Áreas Vinculadas, Buenos Aires, Argentina, 2015.
- [12] International Electrotechnical Commission, Standard IEC 61672-1: Electroacoustics - Sound level meters - Part 1: Specifications, Switzerland, 2013.
- [13] Federico Miyara. *Mediciones acústicas basadas en software*. Editorial: Asociación de Acústicos Argentinos, Rosario (Argentina), 2013.