

# 1 **Call overlapping signals sexual status in Darwin's frogs**

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## 5 **Materials & Methods**

6 We describe patterns of vocal interactions in a social environment conformed by pregnant males  
7 PM), non-pregnant males (NPM) and females (F), recording natural duets between animals  
8 having these three status during the reproductive season lasting five months (October 2015 to  
9 February 2016) in a population located on the Island of Chiloé, Chile (43° 21' S; 74° 6' W). In  
10 addition, we evaluated EVR to playbacks of natural calls of individuals of the three sexual status.  
11 All the behavioural observations conducted in this study were performed in accordance with the  
12 bioethical standards of Facultad de Ciencias, Universidad de Chile

### 13 **Duet recordings**

14 Vocalizations of subjects calling in duets were recorded with a digital recorder (Tascam DR-100)  
15 at a sampling rate of 44.1 kHz and 16-bit resolution and two directional microphones (Sennheiser  
16 ME-66) plugged to each recording channel. The distance separating the two subjects intervening  
17 in the duet was measured and sound pressure level (SPL re 20 µPa, C frequency weighting and  
18 fast time weighting) of calls of one individual conforming the duet was recorded placing a sound  
19 level meter microphone (Extech 407780) adjacent to the tip of the directional microphone.  
20 Latency (registered as phase angle of call onsets between the calls of the two individuals; Klump  
21 and Gerhardt 1992), number of call overlaps and delay between the onset of overlapping calls  
22 between interacting subjects were measured. To discard that call overlap was occurring by  
23 chance between pairs of individuals composing a duet, number of overlaps and overlap delay  
24 between duets was compared using generalized linear models (GLM).

### 25 **Playback experiments**

26 Call bouts of playback stimuli were composed of 10 natural calls of individuals of the three  
27 sexual status having a high signal to noise ratio. The amplitude of call bouts was standardized at  
28 64 dB SPL at the position of the subjects and time intervals between successive calls within a call  
29 bout were generated with random intervals of silence lasting 5 – 60 s. These values approximate  
30 those occurring in natural interactions between individuals of Darwin's frog. Following this  
31 procedure, bouts of calls having different call rates and lasting 138 – 399 s resulted. This  
32 randomization in call timing allowed to evaluate the temporal relationship of the EVR to the  
33 stimuli, independent of potential rhythmic calling behaviour based on an internal oscillator (Zelick  
34 and Narins 1985). Three-minute silent intervals spaced call bouts of the different stimuli and the  
35 order of presentation of call bouts of each sexual status was randomized. Stimuli were presented  
36 with a Samsung J1 WAV player connected via Bluetooth to a portable loudspeaker (i.Sound  
37 5464). Spontaneous vocal activity of the experimental subjects was recorded and thereafter  
38 playbacks of calls of the three sexual status were presented sequentially through a loudspeaker  
39 placed on moss vegetation at 1 m and at an angle of about 90 degrees relative to the focal subject.  
40 Upon completion of each playback experiment, identity of focal individuals was registered.

41 Responses to natural stimuli were analyzed measuring call rate, latency, SPL and number of  
42 overlaps of response calls with the stimuli as for duets. Call rate and SPL were computed for  
43 periods of silence and stimuli presentation, while latency and number of overlaps were measured  
44 only for stimuli presentations. GLM, ANOVA and post-hoc tests were used to compare responses  
45 to stimuli of the three sexual status.  
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