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## Assessment of the calling detection probability throughout the day of two invasive populations of bullfrog (*Lithobates catesbeianus*) in Uruguay

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### ABSTRACT

Bullfrog invasion is a major conservation concern in South America, so there is an urgent need to detect and monitor its many invasion foci. Amphibian sampling methods commonly use calling display, specifically the nuptial calls of males. With the aim of obtaining the better day period to sample and monitor *Lithobates catesbeianus* presence, we recorded its calls at three ponds in two invaded localities in Uruguay (Aceguá, Cerro Largo, and San Carlos, Maldonado) during the reproductive season. Then, we studied the records, obtaining a subsample of calling intensity at the first 5 minutes for each hour. We detected that vocalization intensity remained almost constant between 20:00 and 05:00 h. Detection probability remained high and constant during this period, and then decreased. Therefore, bullfrog displays a constant calling activity during the nights of its reproductive period, even longer than native anurans. This long calling period facilitates its detection during nocturnal sampling.

Key Words: Invasion biology; Bullfrog; Sampling methods; Pond.

Knowing the natural history of an invasive alien species is essential, both to understand its impact and performance in a new environment, and to monitor it (Davis, 2009). The invasion of the American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), in South America is a major concern in conservation for its potential harmful effects on native biodiversity (Laufer *et al.*, 2008; Akmentins and Cardozo, 2010; Nori *et al.*, 2011; Ruibal and Laufer, 2012). One of the most notable attributes of the amphibians is their calling display, specifically the nuptial calls of males (Wells, 2007). *L. catesbeianus* displays a characteristic call, which gives its common name to be similar to the sound emitted by a bull, consisting of a loud call of between three and fifteen sequential vocalizations, with bimodal peaks at 0.2 kHz and 1.4 kHz. Silences periods between 0.5 and 1 second may occur among calls (Capranica, 1968).

Bullfrogs' vocalizations occur over prolonged periods during the warmer months, in which males

can call isolated or in large chorus (Emlen, 1976). The evidence gathered in the northern hemisphere shows that these calls occur during the evening hours, when water temperatures are higher than 20°C and under low wind conditions (Oseen and Wassersug, 2002). Recently, Medeiros *et al.* (2016) have found seasonal differences in the amount of males vocalizing per hour in an invasive population of southern Brazil. While in spring the average number of vocalizing males peaks their maximum between 18:00 and 0:00 pm, in summer this activity peak occurs between 2:00 and 6:00 pm.

Amphibian's vocalization is a useful tool for researchers because of its specificity. Recording males' calling activity during the breeding season is a suitable method for field sampling and monitoring. Sound sampling can be used to assess the distribution and progression of exotic invasive anurans (Heyer *et al.*, 2014). While *L. catesbeianus* calling activity is well known for the northern hemisphere,

**Table 1.** Water bodies where record sampling of bullfrog calls was performed during the sampled season in 2015. In each case the location, date and time of start and end of recording is detailed, and geographic coordinates.

Locality	Sampling Dates	Time Period	Latitude	Longitude
Aceguá, Cerro Largo	9 to 12 of December	17:00 to 14:00 h	31°53'35.5"S	54°09'09.8"W
			31°53'41.2"S	54°09'11.6"W
San Carlos, Maldonado	25 to 26 of December	18:00 to 09:00 h	34°47'11.7"S	54°53'00.7"W

it has barely been studied for invasive populations of South America. The idea of this study was to find the optimal time of the day for detecting this species during the reproductive season in invasive population of Uruguay.

The field surveys were conducted by recording male bullfrog's calling activity in two lentic water bodies in the locality of Aceguá, Cerro Largo Department, and other one in San Carlos, Maldonado Department, in December 2015 (Table 1). In both sites recordings were made throughout the day with Panasonic RR-US310 recorders, located at 30 cm of the water bodies' borders. Registration was performed continuously during the day, with a battery change every 20 hours. Air temperature and relative humidity at the edge of the pond, were recorded by data loggers (Extech RHT10). The same type of data logger, covered and submerged 15 cm, reordered water temperature. These environmental records were performed every 10 minutes.

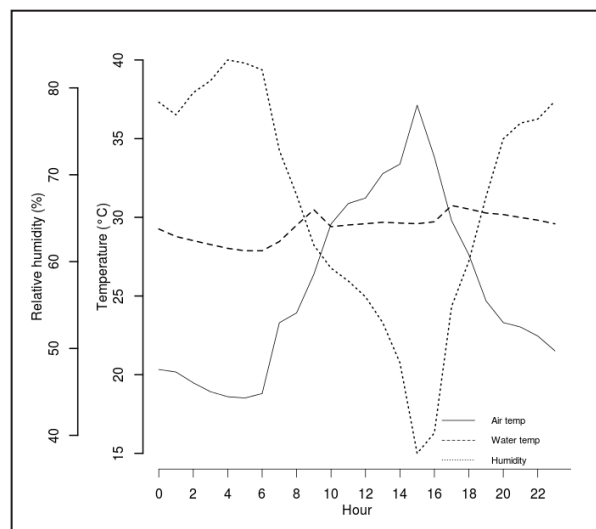
Data analysis was performed with a sub-sample of the record (Table 1). We studied the sonorous records of five contiguous minutes, considering that this time is the minimum visit per point, in a rapid field sampling in search of the presence of bullfrogs. For each hour the first 30 seconds of the 0, 1, 2, 3, 4 and 5 minute was analysed, and the proportion of recordings with bullfrog calls was scored (vocalization intensity). Scores assigned values were between zero and three. Zero was assigned when there was a total absence of calls; one when one third of the 30 seconds record calling males, a score of two when two thirds of the 30 second interval record calls, and three when the entire period of 30 seconds registered bullfrog calls. Thus, six values for each hour interval were obtained, which were added to obtain the average calling intensity per hour and its standard deviation, as well as the environmental variables.

Climatic conditions during the sampling period were among the reported ranges for December in Uruguay (Marengo and Camargo, 2008; Rusticucci and Renom, 2008). Air temperature was around

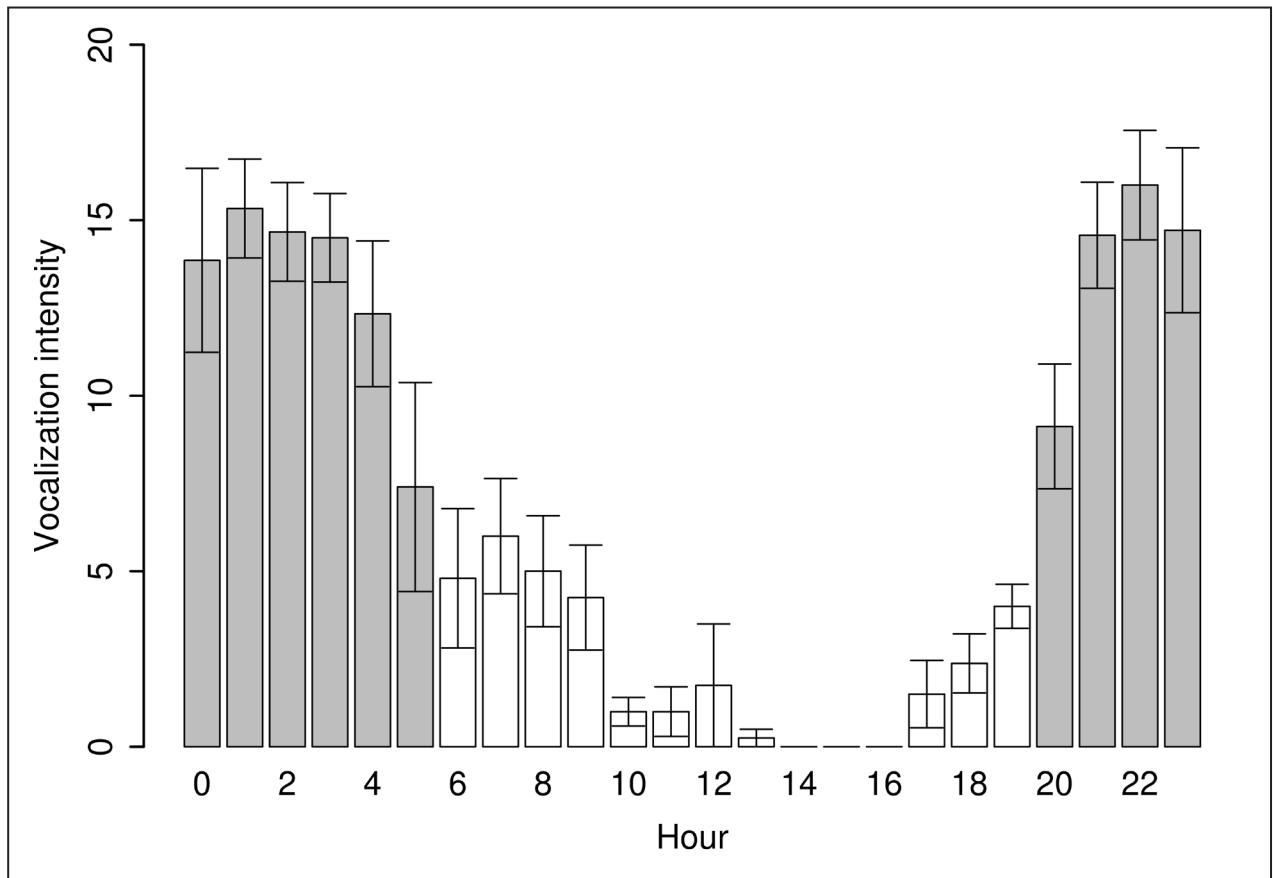
20°C overnight and strongly increased throughout the day peaking at 15:00 hours. Contrary, humidity was higher overnight, falling in the hottest hours. The water temperature remained relatively constant throughout the day with small peaks associated with higher temperature hours (Fig. 1).

Male bullfrog's vocalizations were heard over the day except for a period between 13:00 to 16:00 h. Between 20:00 to 05:00 h vocalization intensity remained relatively constant at the highest levels. After 04:00 h there was a constant decrease tendency in the vocalizations intensities, disappearing completely at 14:00 h. Then at 17:00 h vocalizations started to increase until 20:00 h (Fig. 2).

The pattern observed in Uruguay is similar to that reported for the northern hemisphere (Bridges *et al.*, 2000). Bullfrog male has a long vocalization period spanning practically overnight. Considering native anurans' natural history, it is notable that the period of maximum bullfrog calls is extensive and overlaps with the shorter periods of time used by



**Figure 1.** Records of climatic variables during the sampled period for bullfrog vocal activity in Aceguá, Cerro Largo Department and San Carlos, Maldonado Department. The graph includes means values of air temperature and water, and relative humidity.



**Figure 2.** Mean hour vocalization intensity  $\pm$  1SD of Bullfrog calls in Aceguá, Cerro Largo Department and San Carlos, Maldonado Department, in December 2015. The white bars indicate the daylight hours and the gray bars indicate night hours.

native species (e.g. Bardier *et al.*, 2014). The high density, intensity and duration of *L. catesbeianus* calls could alter the native acoustic niche, affecting native anurans. It has been reported that bullfrog vocalizations can interfere in the acoustic niche of native species of *Hypsiboas* in Brazil. This species alters its calling frequency in the presence of calling bullfrogs, with possible negative consequences on their reproductive fitness (Both and Grant, 2012).

Regarding bullfrog activity sampling periods, we could suggest that better day period to perform species detection by means of vocalizations will be between 20:00 and 05:00 h. We detect that vocalization intensities remained almost constant within this period (Fig. 2). Although during certain times of the day we could record bullfrog vocalizations, the probability of detection declines drastically.

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