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INVASION NOTE

Current status of American bullfrog, *Lithobates catesbeianus*, invasion in Uruguay and exploration of chytrid infection

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Abstract The American bullfrog *Lithobates catesbeianus* is an invasive species that can strongly affect native amphibian communities through competition, predation, or introduction of diseases. This frog has invaded multiple areas in South America, for which niche models predict suitable environments across much of the continent. This paper reveals the state of the invasion of this species in Uruguay and its possible relationship with the chytrid pathogenic fungus, *Batrachochytrium dendrobatidis*. Surveys at invaded sites were conducted from 2007 to 2015, identified two populations undergoing recent range expansion (one of them exponential), two populations that failed to establish, and a new record in an urban area of the capital city, Montevideo. In all the analysed feral populations, chytridiomycosis was found. Our data suggest that the invasion of *L. catesbeianus* in Uruguay is at an early stage, with very localized populations, which might allow for the implementation of cost-effective management plans, with eradication constituting a plausible option.

Keywords Amphibian · Freshwater · Pond · Geographic distribution · *Rana catesbeiana*

Introduction

The bullfrog, *Lithobates catesbeianus* (Shaw, 1802), is a large aquatic anuran native to the eastern part of North America. This frog has been heavily traded for meat production, leading to the establishment of alien populations in temperate and tropical areas worldwide.

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Invasive *L. catesbeianus* generate notorious negative effects on local biodiversity through competition, predation and introduction of diseases (Kraus 2009, 2015), especially the pathogenic fungus *Batrachochytrium dendrobatidis* (Longcore et al. 1999). Over the last decade, several bullfrog populations were reported in the Neotropics (e.g., Laufer et al. 2008; Akmentins and Cardozo 2010; Both et al. 2011; Iñiguez and Morejón 2012); however, the invasion process has rarely been monitored. This has been a constraint for implementation of control and/or eradication programmes of this species in the region (Kraus 2009; Nori et al. 2011a; Speziale et al. 2012).

In Uruguay, this species was introduced in the 1980s, following a production model (closed farms) initially promoted in Brazil (Cunha and Delariva 2009). National aquaculture authorities promoted the establishment of 19 private closed bullfrog farms. This industry did not develop the expected level of business, and by the first years of the last decade all the farms closed without any control of the destiny of their frogs (Laufer et al. 2009).

The first feral population of *L. catesbeianus* in Uruguay was discovered in 2005 at Rincón de Pando, Canelones Department (Laufer et al. 2008). Afterwards, during fieldwork in abandoned frog-farming facilities, two new populations were found at Paraje Bizcocho, Soriano Department, and Aceguá, Cerro Largo Department (Laufer et al. 2009; Ruibal and Laufer 2012). Recently, Lombardo et al. (2016) reported a new water body invaded in San Carlos, Maldonado Department, at a place where no bullfrogs were detected in previous surveys (Laufer et al. 2009).

The assessment of population status and control of bullfrogs is considered a national priority in responding to invasive species present in Uruguay (Aber et al. 2012). The objective of this paper is to report the status of the *L. catesbeianus* invasion from 2005 to 2015, including its geographic extent, annual rate of expansion, population status at the different localities, and extent of chytridiomycosis infection.

Methods

Based on previous information available from a national survey of the 19 bullfrog breeding facilities (Laufer et al. 2008, 2009), a monitoring plan was established for the four sites where bullfrogs were detected in the wild: Rincón de Pando (Canelones Department), Paraje Bizcocho (Soriano Department), San Carlos (Maldonado Department) and Aceguá (Cerro Largo Department). Additionally, we considered the data from another site within Montevideo city from which we gathered information about bullfrog sightings.

At each site, periodic sampling was carried out, on an annual basis. Every year, since bullfrog detection, we performed a survey during the reproductive season (Laufer et al. 2017). It consisted of intensive searches for adults, eggs and larvae in all water bodies present within a 1 km radius of each record. In those cases in which feral bullfrogs were found, concentric sampling was done around the facilities of the frog farms (primary focus) until reaching at least 1 km beyond the last record of bullfrog individuals. Sampling was carried out using two methods: fishing for tadpoles with hand nets and trawls, and detection of postmetamorphic individuals by sighting and/or listening for adult nocturnal vocalizations during the breeding season (Dodd 2010). Net sampling consisted of two sweeps of about 7–10 m per body of water, and adult samplings consisted of three consecutive nights of slow-paced walking around the perimeter of the water bodies by three specialized surveyors, which is sufficient for an extremely conspicuous species such as the bullfrog. We also interviewed local residents about the occurrence of feral bullfrogs. This local information was always checked against our field data. Bullfrog spatial distribution was mapped using the field records with the aid of a geographic information system.

For the locality Aceguá, for which there is an accurate annual record of the stage of the invasion since 2007, population growth rate based on the number of invaded water bodies was estimated. In order to do this, a generalized linear model of the Gaussian family was used with the logarithmic connection function. The year was used as the independent variable, and the accumulated number of invaded ponds as the dependent variable (Zuur et al. 2007).

Survey for chytrid

The diagnosis of infection by amphibian chytrid fungus (*B. dendrobatidis*) was tested on feral specimens from all sites in which they were present. We used both conventional PCR (Annis et al. 2004) and RT-PCR (Boyle et al. 2004) to analyse skin swabs (MW113, Medical Wire and Equipment) taken from live adult bullfrogs in the field to detect the presence of chytrid. The swabbing procedure was standardized, rubbing the swab five times on the ventral surface of the body, hands and feet of frogs. In the case of larvae, oral-disc swabs were performed. Swabs were preserved in ethanol at −20 °C until processed, and the positive control used was a DNA sample of the strain JEL423. Negative controls were water purified in Milli-Q systems, further treated with UV radiation, and amphibian DNA was extracted from liver samples. Diagnoses were additionally confirmed at all localities by identification of fungi on hematoxylin- and eosin-stained histological sections of adult frog skin and oral discs of tadpoles, following Berger et al. (1999). As an additional control, some of the DNA samples of the PCRs were sequenced (Borteiro, unpublished data).

Results

Feral bullfrogs were detected in all the explored sites. Most of these populations occur in rural areas, except for the records within Montevideo city, at Instituto de Investigaciones Biológicas Clemente Estable (IIBCE, 34°53′14,4″S; 56°08′33,3″W). In this institute, bullfrog calls were heard and some individuals were photographed on three occasions between 2014 and 2015 (Fig. 1a). They were first detected in 2005 in Costa de Pando (Fig. 1d), then in 2007 in Paraje Bizcocho (Fig. 1b) and Aceguá (Fig. 1c), and finally in 2015 in San Carlos (Fig. 1e; Laufer et al. 2008, 2009; Lombardo et al. 2016; Ruibal and Laufer 2012). The Montevideo record, which has not been reported previously in the literature, is a single artificial water body within the facilities of the IIBCE.

Currently, the only persisting and expanding populations are the ones of Aceguá and San Carlos. Negative results at Costa de Pando agree with information given by local inhabitants, who told us that in the years before our initial survey (in 2005) bullfrogs were much more abundant (34°44′20″S; 55°55′30″W; see Laufer et al. 2008). In our subsequent visits to this site, from 2007 to 2015, we could not detect any evidence of bullfrogs, which accounts for approximately a 10-year period without reports of the species there (Fig. 1d). A similar situation is seen at Paraje Bizcocho, where a population was detected in 2007 and 2008 (33°27′55″S; 58°10′08″W), but in subsequent surveys (2009–2015) we found no evidence of its presence. The survey site at this locality is close to Bizcocho Stream and the San Salvador River, an area that has been highly altered by establishment of soybean cultures since 2008 (Fig. 1b).

Although bullfrogs were not previously detected by us in San Carlos during the 2007 surveys (see Laufer et al. 2009), the species was likely present given that a large bullfrog farm was formerly active there, and local residents indicated commonly finding the species in previous years (34°47′03″S; 54°53′42″W). Recently, Lombardo et al. (2016) reported the presence of an invaded pond in the area (34°47′03″S; 54°53′42″W), and in our field sampling in 2015 we observed that it was more widespread, actually involving six nearby water bodies. All belong to small rural establishments dedicated to cattle production and recreation farms, near the National Route 9 and San Carlos Stream (Fig. 1e).

A very different situation was observed at Aceguá, (Fig. 1b). This locality is within a hilly area, a landscape dominated by native vegetation, where the bullfrog has mostly invaded artificial water reservoirs used for cattle watering (31°53′36″S; 55°09′26″W). From its first detection in 2007 until 2011, annual surveys have indicated that this population is restricted to eight water bodies. Four are relatively large man-made ponds associated with a slaughterhouse that has been inactive since at least 2005. In 2012, we detected five newly invaded water bodies. Then, the dispersal of bullfrogs remained constant until our last surveys, currently including at least 23 water bodies. It is interesting to note that we recorded an unexpected upland dispersal towards Aceguá town.

Analysing the dynamics of the bullfrog invasion in Aceguá over time, we found that the population size (estimated by the number of invaded ponds) significantly fits an exponential model (explained deviance 93.87%, $P < 0.001$, fd = 1). We could observe that during the first sampling years (2007–2011), the population size remained constant, but exponential

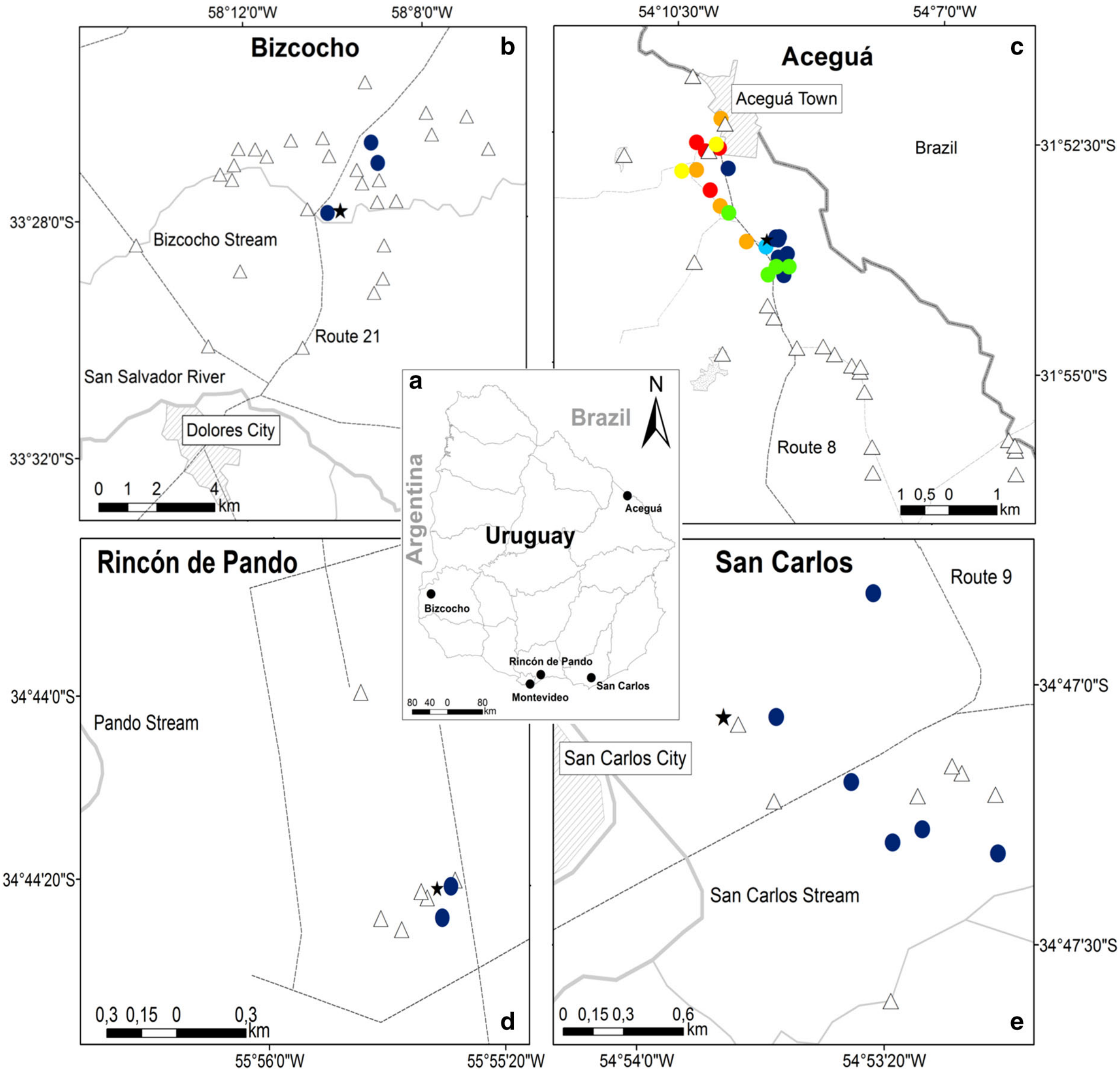

Fig. 1 Bullfrog, *Lithobates catesbeianus,* invasion foci in Uruguay. In the central map of Uruguay, the five invaded sites are noted with *black circles* (Soriano, Canelones, Montevideo, Maldonado and Cerro Largo) (**a**). *Solid dots* indicate the presence of feral specimens in water bodies at each study site (**b–e**), and *triangles* correspond to water bodies without the species during the study period (2005–2015). Locations of abandoned bullfrog farms are marked with *stars*. In Paraje Bizcocho (**b**) ponds invaded in 2007 are indicated with *black circles*, but from 2009 to 2015 they were not detected again. In Aceguá (**c**), the sequential invasion of water bodies recorded in 2007, 2011, 2012, 2013, 2014 and 2015, are indicated with *blue*, *sky-blue*, *green*, *yellow*, *orange*, and *red* 2015. In Rincón de Pando (**d**) the invaded ponds in 2005 were apparently free of bullfrogs from 2007 to 2015. In San Carlos (**e**) bullfrogs were detected in 2015 in water bodies previously surveyed in 2007. *Dotted lines* indicate routes and paths; *continuous lines* indicate watercourses and *thick continuous lines* correspond to national borders. Urban centres are represented by lined polygons

growth has been observed since 2012. This model predicts that the population will continue to expand, reaching by 2020 to include approximately 50 water bodies (Fig. 2) .

Chytrid

Batrachochytrium dendrobatidis was verified to be present in all studied sites, except for Montevideo, where no chytrid swabs samples were obtained. Chytrid-positive samples were detected by both histological and molecular analyses (Table 1).

Discussion

The invasion scenario of bullfrogs in Uruguay differed among reported populations. In some cases populations failed to establish, whereas others show expansion after 10 years. This early detection of an invasion, as well as its detailed mapping, are infrequently available for other biological invasions in South America (Speziale et al. 2012). The reported on-going bullfrog invasion is alarming regarding to its possible deleterious effects, but there is also some cause for encouragement. The persistence of two established populations of *L. catesbeianus* is of great concern, given some predictions for high niche suitability in the country (Nori et al. 2011b). However, the early stage of the invasion in certain restricted geographic areas (less than 6 km^2 in Aceguá and 1 km^2 in San Carlos) provides a unique opportunity for successful eradication. Rapid control measures are known to improve likelihood of eradication, as seen with feral bullfrogs in England and Germany and *Xenopus laevis* in the United States (reviewed by Kraus 2009). A rapid intervention in Uruguay that included multiple forms of removal, drying of water bodies, and attempts to isolate expanding populations would preclude dispersal, with high chances of achieving eradication. It should be noted that the invasion front of bullfrogs in southern Brazil is relatively close to the population in Aceguá (Both et al. 2011), but this does not justify the current failure to implement control measures by Uruguayan agencies.

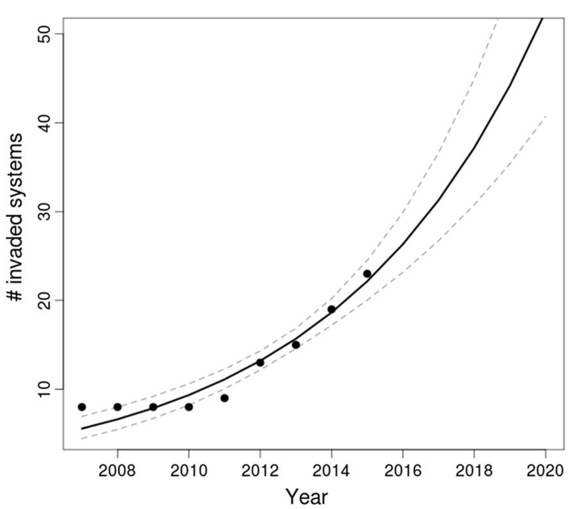

Fig. 2 Dynamics of the bullfrog invasion in Aceguá, northwestern Uruguay, observed through the number of invaded water bodies. The *solid line* indicates the trend expected by the exponential model (# invaded aquatic systems $= 1.9 \times 10^{-150} \times 1.19$ year; explained deviance 93.87%, $P < 0.001$, fd $= 1$), and *grey dotted lines* the 95% CI. The estimated projection by the model is presented until 2020

The population of Aceguá is a management priority as it is undergoing exponential expansion. The situation in the San Carlos population seems to be different, as the species, reappearance alerts us to possible population oscillations in the early stages of invasion, which should be taken into account in monitoring programs (Lockwood et al. 2006). This fact suggests that long-term monitoring would be needed to confidently assess the invasion status at sites where the species seems to have vanished, like Rincón de Pando and Paraje Bizcocho, and the remaining 17 initially sampled farms (see Laufer et al. 2009). Besides, the detection of free-ranging bullfrogs at a research institute in Montevideo exemplifies the poor knowledge of the risks associated in using invasive species as laboratory research models. Furthermore, the invasion of *X. laevis* in Chile seems to have also originated due to negligence by a research institute (Lobos and Jaksic 2005).

As reported for elsewhere (Garner et al. 2006), bullfrog populations in Uruguay are infected with *B. dendrobatidis*. This pathogen has been linked to the global decline of amphibians, and exotic anurans like bullfrogs are considered epidemiologically relevant vectors. It is then important to assess the occurrence of different strains of amphibian chytrids present in the invaded zones and their possible effects on native species (Schloegel et al. 2012).

Finally, the importance of establishing a national monitoring programme of biological invasions must be emphasized as a key element for their management at national and regional scales (Latombe et al. 2016). Considering the evidence presented in this work and the biological risks associated with bullfrog invasions, governmental agencies in Uruguay should implement the monitoring and control of this alien invasive species. Eradication of the small reported populations is still feasible, and the geo-referenced information presented here is a key tool for this porpoise. In this

Table 1 Evidence of *Batrachochytrium dendrobatidis* infection in feral bullfrogs *Lithobates catesbeianus* from Uruguay

Locality	Date	Analysed individuals	Detection technique	Positive N
Rincón de Pando	2005	1 larva	Histology	1
Bizcocho	2007	1 larva	Histology	1
Aceguá	2007	2 larvae	Histology	2
Aceguá	2012	10 adults	Conventional PCR	1
San Carlos	2015	10 larvae	RT-PCR	10

scenario, coordination with stakeholders such as research centres, local governments and conservation NGOs is fundamental. Proper management of *L. catesbeianus* in Uruguay would help to suppress the invasion and would be a good example of rapid response to a serious environmental threat.

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