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Rapid Communication

New record of a feral population of *Lithobates catesbeianus* Shaw, 1802 in a protected area (Santay Island) in the Ecuadorian coast

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Abstract

The American bullfrog (*Lithobates catesbeianus* Shaw, 1802), an amphibian species native to eastern North America, is considered one of the 100 most harmful invasive species in the world. Previous studies document several feral populations in the Amazon and Andean regions of Ecuador. However, only few adults have been reported in the Coast region, despite some evidence suggesting its introduction 31 years ago. Using visual and auditory cues, we explored a 490-hectare wetland area at Santay Island, a protected sanctuary and a Ramsar site on the Ecuadorian Coast. Bullfrogs were detected in seven out of 15 sampled ponds in all types of habitats except for mangroves. The low abundance of adults and juveniles suggests a recently established population. This is the first record of a feral population inside a protected area or Ramsar site in Ecuador. In accordance with the Ramsar Convention mission of preserving wetlands, we propose two strategies to manage bullfrogs at Santay Island.

Key words: American bullfrog, amphibian conservation, invasive species, management strategy, wetlands

Introduction

The American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), is an invasive species, native to the eastern North America (Wright and Wright 1933; Dickerson 1969; Bury and Whelan 1984). This aquatic anuran lives mainly in shallow permanent water bodies and has the capacity to thrive in human-altered areas (Moyle 1973). *Lithobates catesbeianus* has a large body size, generalist diet, vast natural range, high population density, and easily invades ecosystems (Stumpel 1992; Pearl et al. 2004; Kiesecker and Blaustein 2008). The American bullfrog can negatively impact the local diversity of ecosystems it invades (D'Amore 2012) through predation (Moyle 1973; Kiesecker and Blaustein 1997; Pearl et al. 2004), competition

(Moyle 1973; Kiesecker et al. 2001; Wu et al. 2005), and transmission of pathogenic organisms (Hanselmann et al. 2004; Galli et al. 2006; Garner et al. 2006; Schloegel et al. 2009; Martel et al. 2012). Bullfrogs have been linked to several population declines and local extinction of native amphibians of places it has invaded (Fisher and Shaffer 1996; Kiesecker and Blaustein 1997; Kats and Ferrer 2003; Da Silva et al. 2011; Laufer and Gobel 2017; Gobel et al. 2018). Numerous studies have demonstrated significant negative correlations between American bullfrogs and native amphibian's populations (Moyle 1973; Kiesecker and Blaustein 1997; Lawler et al. 1999; Kiesecker et al. 2001; Pearl et al. 2004; Wu et al. 2005). All these precedents have led the IUCN to recognize the American bullfrog as one of the top 100 world's worst invasive species (Lowe et al. 2000).

The American bullfrog was introduced and established feral populations in most continents (Green and Campbell 1984; Stumpel 1992; Borges-Martins et al. 2002; Casas-Andreu et al. 2002; Hirai 2004). It is mainly farmed as a protein source for human consumption, but it has also been released as a biological control agent or as an ornamental species (Jennings and Hayes 1985). In South America, it was introduced to Brazil (Both et al. 2011), Colombia (Rueda-Almonacid 1999), Ecuador (Cisneros-Heredia 2004; Cobos et al. 2015; Valarezo-Aguilar et al. 2016), Peru (Catenazzi and von May 2014), Uruguay (Laufer et al. 2008), Argentina (Sanabria et al. 2011) and Venezuela (Hanselmann et al. 2004).

In Ecuador, bullfrogs were first introduced to the Guayas province in 1988 (Ecuadorian Coast), with the import of 100 adults and 70,000 tadpoles for commercial purpose (Casares 1992; Velasco 2001). During the 90's, twenty three farms were established in Ecuadorian Amazonia (sixteen in Zamora Chinchipe, five in Napo, two in Pastaza and two in Morona Santiago) and; six farms in the Ecuadorian Coast (four in Guayas and two in Los Ríos) (Villacis and Zurita 2002). In Amazonia, feral populations of this exotic species were reported nearby an abandoned bullfrog farm in Napo province (Cisneros-Heredia 2004) and in a biological station (CEDAMAZ) located in the Zamora Chinchipe province (Valarezo-Aguilar et al. 2016). In Andes, a feral population of bullfrog was reported in a recreational park in Loja city (Loja province; Cobos et al. 2015). Only few individuals of this species have been reported in the northern Ecuadorian Coast (i.e. at Bahía de Caráquez; Cisneros-Heredia 2004; Cobos et al. 2015). These records confirm that bullfrogs are escaping and establishing feral populations in Ecuador.

Santay Island, a Ramsar site since 2000, is one of the few protected areas in Guayas province in the Ecuadorian Coast. Presence of the American bullfrog at Santay Island was suggested in a report by the Ministry of the Environment of Ecuador in 2015 (Wilver Bravo *personal observation*) and by a photographic field guide by Zavala-Becilla (2017). However, the establishment of breeding populations in the island is yet unconfirmed.

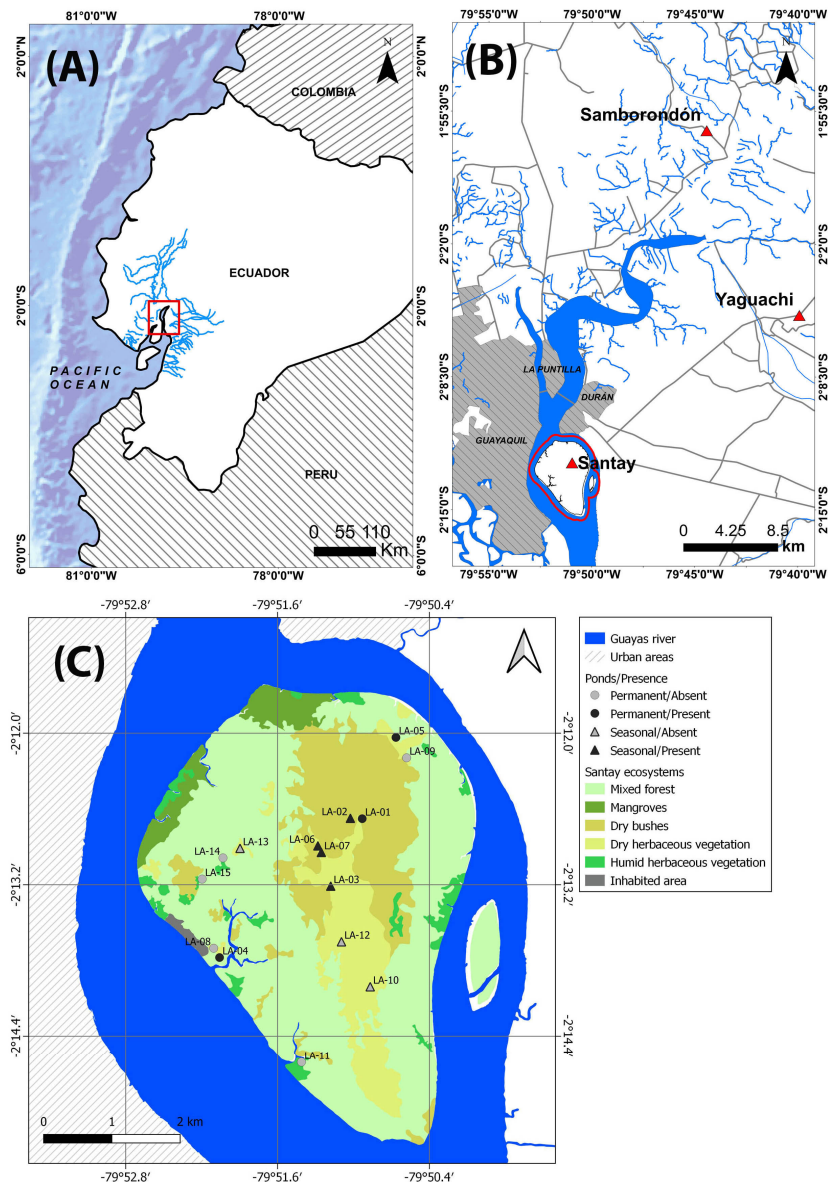


Figure 1. A) The sampling location and peripheral records of American bullfrog in Ecuadorian Coast. B) The sampling location (Santay Island) in the Guayas river system and closest peripheral records of American bullfrog. C) Presence and absence of American bullfrog in surveyed permanent and seasonal ponds at Santay Island.

The main goal of this study was to explore Santay Island for the presence of breeding populations of *L. catesbeianus* and determine its distribution within this protected area. Also, we obtained a list of the local amphibian assemblages for ponds with presence and absence of the American bullfrog. We propose management strategies to control the current populations and prevent further expansion invasion in Santay Island.

Materials and methods

Study area

Santay Island is a sedimentary formation located between the delta and the basin of the Guayas river, 800 m east from the city of Guayaquil (02°13'S; 079°51'W; altitude: 0 to 10 m a.s.l.) in Guayas province, Ecuador (Figure 1).

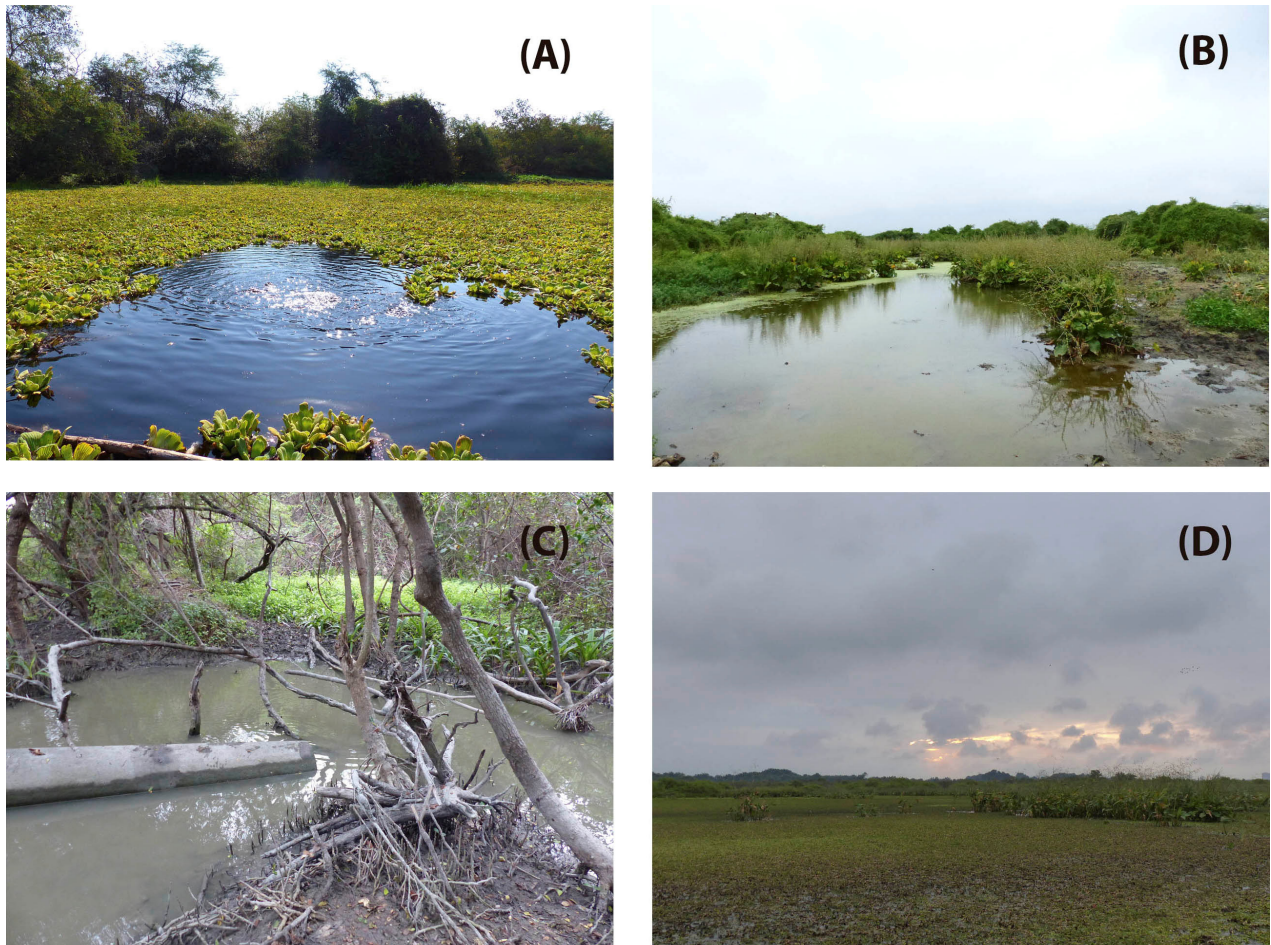


Figure 2. Water-bodies surveyed at Santay Island: (A) artificial and permanent, (B) natural and seasonal, (C) natural and permanent, and (D) natural and seasonal. Photos by Carlos Cruz-Cordovez and Kimberly Rizzo.

Mean annual temperature is 27 °C and mean annual precipitation is 1 650 mm (Herrera et al. 2018). The ecosystem consists of 2 179 hectares of diverse wetlands originating from the river flooding during wet season (Figure 2).

Water availability at Santay Island is mostly determined by seasonal changes in rainfall. During the wet season (January–April), the island is flooded, ponds reach their maximum water level and seasonal ponds, absent during the dry season, emerge. As a result, bullfrogs spread and increase their distribution. During the dry season (May–December), in contrast, most natural ponds dry out. Only a few ponds subject to tidal influence are able to persist in the absence of rains (i.e. LA-14 and LA-15). However, water levels of most artificial ponds do not vary with the natural hydrology of the area. Six out of eight artificial ponds persist throughout the year regardless of the tides (i.e. LA-01, LA-04, LA-05, LA-08, LA-09 and LA-11) (Figure 2).

Native amphibian assemblages include *Rhinella horribilis*, *Scinax quinquifasciatus*, *Trachycephalus jordani*, *Trachycephalus quadrangulum* and *Leptodactylus labrosus* and *Engystomops pustulatus* (Zavala-Becilla 2017).

Field surveys

We sampled a 490 hectare-area including 15 ponds. One-night surveys (18:00–24:00 h) were conducted weekly at each pond from May to August 2018. During surveys, 20 × 2 m linear transects along the shorelines were travelled five times to maximize the chances of detecting bullfrogs and other amphibian species (Liu et al. 2015). To maximize the chances of detecting tadpoles and egg masses, we increased the sampling effort in permanent ponds. Several studies indicate that bullfrogs need permanent waterbodies for breeding (e.g. Fuller et al. 2010; Cook et al. 2013). Detection was carried out auditory and visually. The first allows for detection of calling males during the breeding season, when sampling was carried out. Some bullfrogs detected visually were collected by hand or using scoop nets. These were placed in separate plastic containers and euthanized via transdermal exposure for 10 min to benzocaine (3 g.L⁻¹; Dodd 2008). We measured the snout-vent length (SVL; to the nearest 0.02 mm) and body mass (to the nearest 0.01 g) of each bullfrog. To characterize each pond, we recorded the presence/absence of aquatic vegetation inside each pond. We also classified the vegetation surrounding each pond as: (i) mangrove, (ii) forest, (iii) scrubland, (iv) herbaceous or (v) bare soil. We recorded pond hydroperiod (permanent or seasonal), depth (shallow < 1.5 m or deep > 1.5 m for permanent ponds; variable for seasonal ponds) and origin (natural or artificial).

To compare the local anuran assemblages between ponds with and without the presence of bullfrogs, native amphibians were surveyed using the same transects used for bullfrogs. Most native anurans at Santay Island are terrestrial species, but all use waterbodies for breeding or feeding.

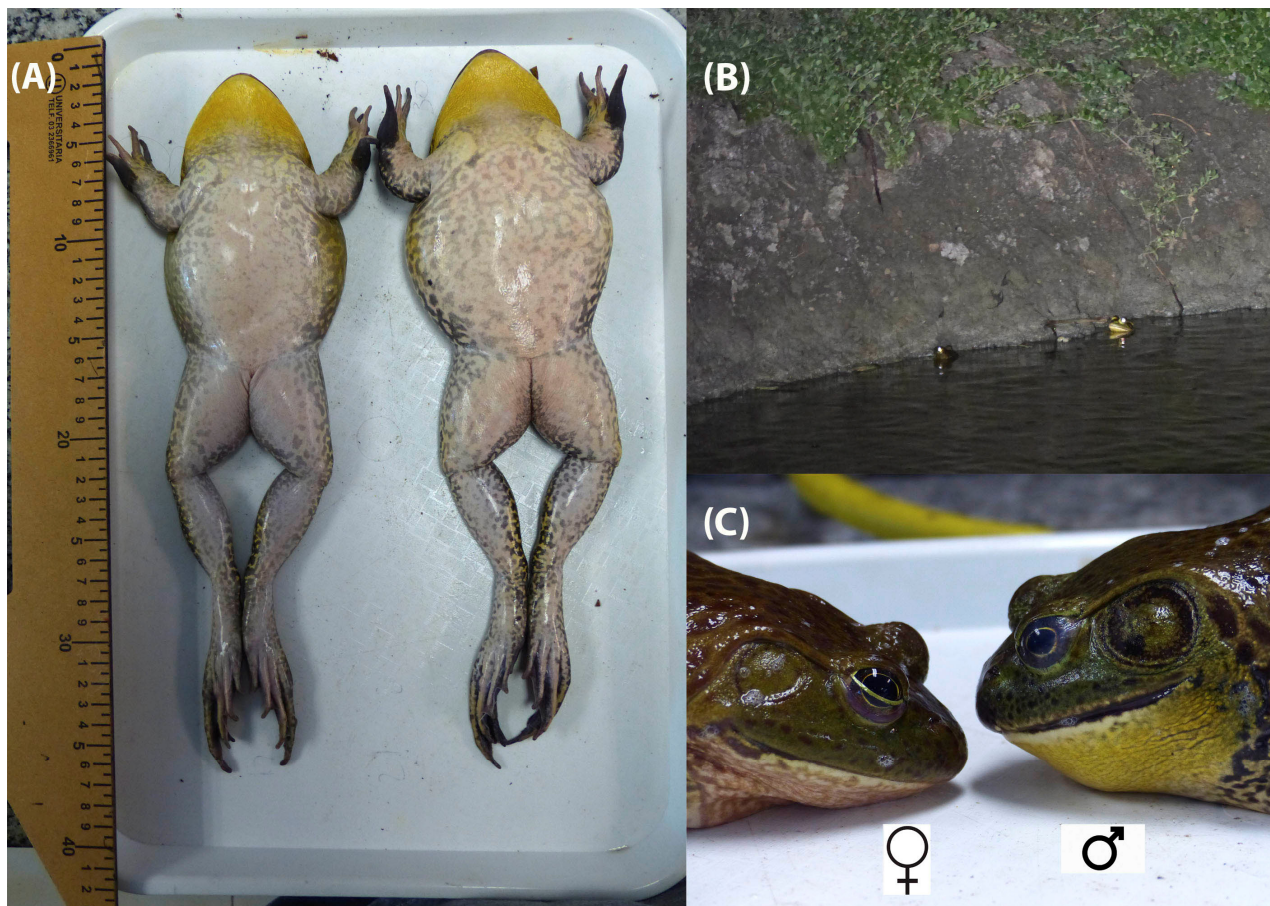
Results

American bullfrogs were detected in seven out of 15 ponds. We sighted two juveniles in LA-05, thirteen adults in LA-01, and four adults in LA-04. These three ponds are permanent, thus, had the highest chance of having bullfrogs. Also, adult males were heard calling from natural seasonal ponds (LA-02, LA-03, LA-06, and LA-07) and from artificial permanent ponds (LA-01, LA-04 and LA-05) (Figure 1). Almost all invaded ponds had aquatic vegetation but, conversely, most ponds without aquatic vegetation showed no evidence of bullfrogs (Table 1). Also, we did not detect bullfrogs in ponds surrounded by mangroves (Table 1).

A total of seven adult American bullfrogs, four males and three females, were collected from ponds LA-01 and LA-05. SVLs for captured males ranged from 122.00 to 179.00 mm (mean ± SD: 159.78 ± 25.67 mm), and from 166.00 to 172.00 mm (mean ± SD: 168.67 ± 3.06 mm) for captured females (Figure 3).

Table 1. Some characteristics of the 15 ponds sampled at Santay Island, indicating the presence or absence of American bullfrog.

ID-Pond	Presence/ Absence	Origin	Seasonality	Profundity	Type	Aquatic Vegetation	Vegetation around
LA-01	Present	Artificial	Permanent	Deep	Lentic	Yes	Forest
LA-02	Present	Natural	Seasonal	Variable	Lentic	Yes	Scrubland
LA-03	Present	Natural	Seasonal	Variable	Lentic	Yes	Herbaceous
LA-04	Present	Artificial	Permanent	Deep	Lentic	No	Herbaceous
LA-05	Present	Artificial	Permanent	Deep	Lentic	Yes	Forest
LA-06	Present	Natural	Seasonal	Variable	Lentic	Yes	Herbaceous
LA-07	Present	Natural	Seasonal	Variable	Lentic	Yes	Herbaceous
LA-08	Absent	Artificial	Permanent	Deep	Lentic	No	Forest
LA-09	Absent	Artificial	Permanent	Deep	Lentic	No	Forest
LA-10	Absent	Artificial	Seasonal	Deep	Lentic	No	Bared Soil
LA-11	Absent	Artificial	Permanent	Deep	Lentic	No	Mangrove
LA-12	Absent	Natural	Seasonal	Variable	Lentic	Yes	Scrubland
LA-13	Absent	Artificial	Seasonal	Shallow	Lentic	No	Mangrove
LA-14	Absent	Natural	Permanent	Shallow	Lotic	No	Mangrove
LA-15	Absent	Natural	Permanent	Shallow	Lotic	No	Mangrove


Figure 3. (A) Adult American bullfrog specimens collected at Santay Island, (B) Invasive American bullfrog in pond LA-01 at Santay Island, and (C) Sexual dimorphism (throat colouration and tympanum diameter). Photos by Carlos Cruz-Cordovez and Felipe Espinoza.

Six native amphibian species were detected in the island. Five were seen in ponds with and without American bullfrogs (*Scinax quinquefasciatus*, *Engystomops guayaco*, *Leptodactylus labrosus*, *Trachycephalus quadrangulum*, and *Trachycephalus jordani*) and only one (*Rhinella horribilis*) was never found in ponds with bullfrogs.

Discussion

Previous accounts of *L. catesbeianus* were restricted to the Amazon (Napó and Zamora Chinchipe), the Andean (Loja) and, the Coast (Manabí) regions of Ecuador (Cisneros-Heredia 2004; Valarezo-Aguilar 2012; Cobos et al. 2015). This new record from Santay Island, in the outermost part of the Guayas river basin, is the first for a feral population inside a protected area or Ramsar site in Ecuador.

The low abundance of bullfrogs observed at Santay can be attributed to the presence of a sink population, long-established but persisting due to the sporadic immigration of adults from upstream source populations. Some populations in wetland ecosystem of the Pacific Northwest appear to act as sink populations because bullfrogs are unable to complete metamorphosis in ephemeral or drained wetlands (Cook et al. 2013). Alternatively, the population at Santay could be an incipient breeding population recently established. Several observations favor the second hypothesis over the first one. Although this species was introduced into the Guayas river basin 31 years ago, only recently, have individuals been sighted within the sanctuary or nearby. In 2018, 77 adults were collected from a fish farm 17 km upstream from Santay Island (Mero 2018) and few individuals were sighted 5 km upstream (iNaturalist.org, <https://www.inaturalist.org/observations/27970963>) (Figure 4). Bullfrogs are unlikely to go undetected for long periods because their larvae and adults are conspicuous, and males have loud distinctive advertisement calls that can be heard at a distance. Park rangers at Santay Island never heard this frog before 2015 (Wilver Bravo *personal communication*). Recent logging of mangroves for development projects possibly facilitated bullfrog access to this island. In the past, Santay was almost entirely surrounded by mangroves, the only habitat where we never recorded bullfrogs. If mangroves act as a deterrent for bullfrogs, it is possible that they prevented bullfrogs from colonizing the island in the past, and only with the recent clearing of some of this vegetation have bullfrogs been able to access the island. Larger scale studies based on occupancy models will be necessary to determine if the observed negative correlation between mangrove and bullfrog holds at different geographic scales. On the other hand, the presence of juveniles suggests that bullfrogs are breeding in this sanctuary. While juveniles can and do disperse from their natal areas, they tend to move between nearby ponds (Willis et al. 1956), and the closest known bullfrog population to this sanctuary is 17 km away. At Santay Island, artificial permanent water reservoirs from past agricultural practices, known to play an important role in the establishment of bullfrogs elsewhere (Cook and Jennings 2007; Fuller et al. 2010; Cook et al. 2013; Peterson et al. 2013), could have facilitated breeding.

Several Species Distribution Models (SDMs) have identified the Guayas river basin as climatically suitable for *L. catesbeianus* (Nori et al. 2011; Iñiguez

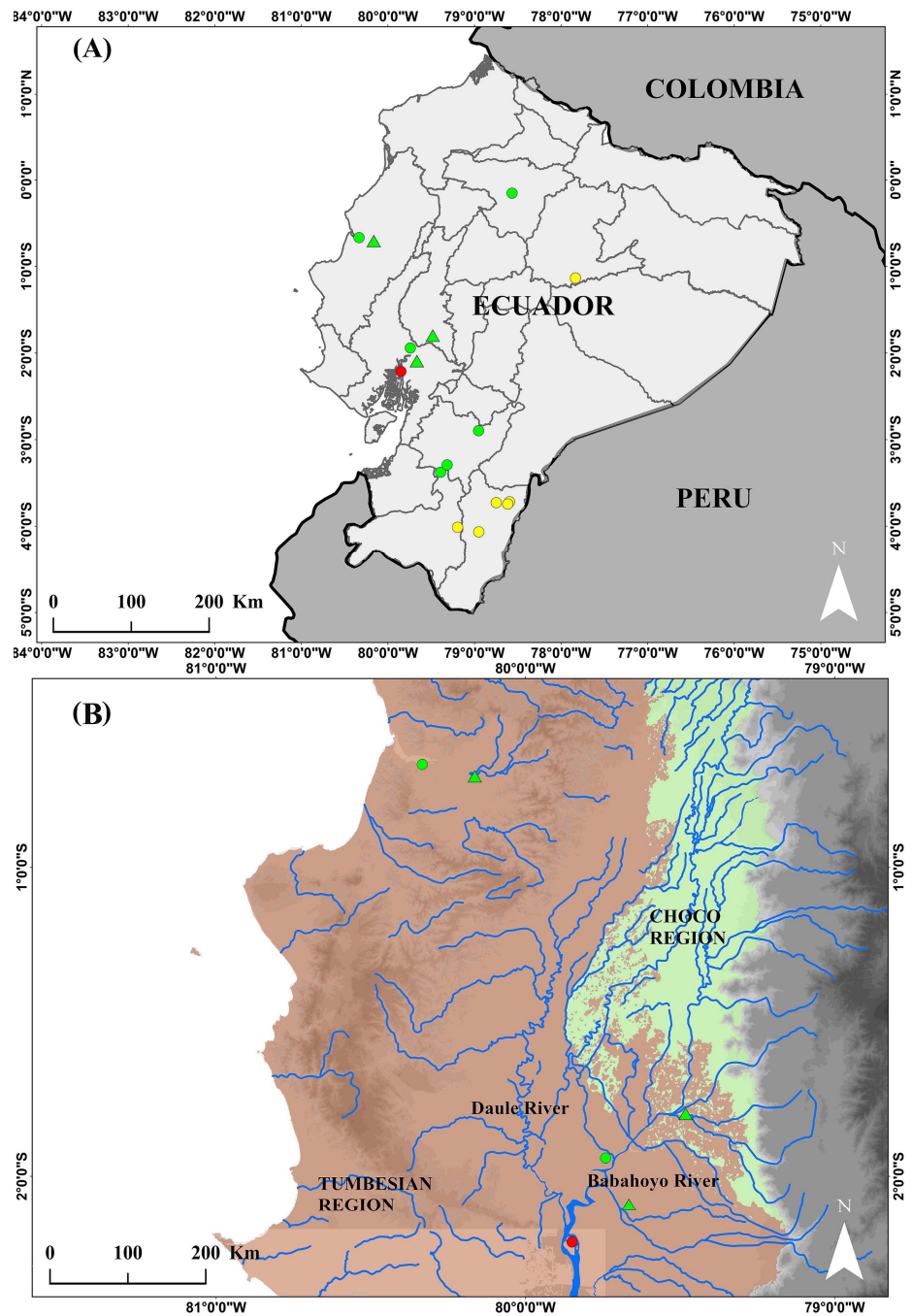


Figure 4. Occurrences of American bullfrog in Ecuador (A) and detailed distribution of the species in Ecuadorian Coast (B). Records in red circle represent the new location reported in this study, yellow circles correspond to confirmed occurrences of feral population published in the literature, green circles correspond to records available in non-formal sources, and green triangles represent records of the species in aquaculture farms. Coordinates of the occurrences are showed in Supplementary material (Table S1).

and Morejon 2012). Also, characteristics present in Guayas river basin, such as, water permanency, seasonal ponds, low elevation and the presence of agricultural activities have been previously correlated with bullfrog presence in other invaded areas (Moyle 1973; Conant 1975; Bury and Luckenbach 1976; Ficetola et al. 2007; Gahl et al. 2009; Fuller et al. 2010; Peterson et al. 2013; Sepulveda et al. 2015). Although seasonal ponds can often act as stepping stones for their dispersal in wetland ecosystems (Gahl

et al. 2009), bullfrogs more often depend on permanent ponds for long-distance migration (Ficetola et al. 2007; Fuller et al. 2011; Sepulveda et al. 2015). Thus, artificial water bodies can significantly increase the dispersal rates of this invasive species (Youngquist and Boone 2014). Guayas basin is an important rice growing and aquaculture area with many artificial ponds and irrigation channels. These artificial water reservoirs allow bullfrogs to breed and complete their development during the dry season when most natural ponds disappear. A possible establishment of bullfrog populations in the Guayas basin could potentially affect aquaculture by tadpole infestations causing economic and production losses. For example, in Florida the net fish production losses due tadpole infestation was estimated at \$ 1.78 million per year (Kane et al. 1992). On the other hand, Guayas river basin is an important pathway corridor between the Choco and Tumbesian ecoregions (Figure 4). Although a global SDM did not classify these ecoregions as climatically suitable for *L. catesbeianus* (Ficetola et al. 2007; Nori et al. 2011), ongoing anthropic modifications of the landscape may facilitate its establishment in these regions, where endemism is high (Mittermeier et al. 2011). It is necessary to identify the front of invasion of bullfrogs and its rate of expansion in this corridor.

At Santay Island, bullfrogs were found in sympatry with native amphibian tadpoles. Bullfrog impact on these native amphibians is not known, but it is possible that they predate on or compete with some of them (e.g. Moyle 1973; Kiesecker and Blaustein 1997; Pearl et al. 2004; Kiesecker et al. 2001; Wu et al. 2005). Only *Rhinella horribilis* was never observed in ponds with bullfrogs. Given the tendency of both species to inhabit a wide range of ecosystems (Bionda et al. 2011), it is likely that an avoidance mechanism exists on one of these species.

Given Ramsar Convention mission to conserve wetlands and the capability of bullfrogs of altering entire ecosystems, it is a priority to reverse the invasion of bullfrogs at Santay Island or, alternatively, to minimize its impact on this ecosystem. Following Maret et al. (2006), we propose a two-method strategy for reducing the densities or extirpating the population of *L. catesbeianus* at Santay Island. The first method aims to reduce reproduction and interfere with development by drying up the few available artificial permanent ponds and manually removing individuals during the dry season. This method has been suggested to control bullfrog populations (e.g. Maret et al. 2006; Boone et al. 2008; Fuller et al. 2010). The second method focuses on reducing the flow of propagules into Santay Island by controlling the abundance of individuals in source populations located upstream. A systematic survey along the right shorelines of Guayas and Babahoyo rivers is necessary for identifying the major sources of colonizers.

During the sampled period, there were methodological restrictions related to the landscape, site access, and vegetation. Sampling during the wet season, when bullfrogs tend to breed, maximizes the chances of finding

eggs and tadpoles, but reduces visual detection rates of juveniles and adults. Therefore, future investigations to estimate the abundance of juveniles and adults of bullfrog should be carried out during the dry season at Santay Island.

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Ethics and Permits

This project was realized with number permit 027-2017-IC-FLO/FAU-DPAG/MAE granted by Environment Ministry of Ecuador. All research pertaining to this article did not require any approving ethics committee.

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Supplementary material

The following supplementary material is available for this article:

Table S1. Coordinates of occurrences of American bullfrog in continental Ecuador.

This material is available as part of online article from:

http://www.reabic.net/journals/bir/2020/Supplements/BIR_2020_Cruz-Cordovez_etal_Table_S1.xlsx