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INTRODUCTION OF THE AMERICAN BULLFROG *LITHOBATES CATESBEIANUS* (ANURA: RANIDAE) IN NATURAL AND MODIFIED ENVIRONMENTS: AN INCREASING CONSERVATION PROBLEM IN ARGENTINA

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Abstract. Several previous studies have established that the introduction of the American bullfrog, *Lithobates catesbeianus,* produces negative effects on native communities. Herein, the American bullfrog is reported for the first time in the province of Buenos Aires, Argentina in the locality of 9 de Julio. The stomach contents of the collected specimens were examined and the reproduction of the species was also confirmed at the study area. A total of 85.7% (n = 30) of the analyzed stomachs contained prey items, and eleven of these were identified. The prey items belonged mostly to coleopterans (50%) and vertebrates (11%). The pathogen *Batrachochytrium dendrobatidis* was not found on skin samples examined. The installation of *Lithobates catesbeianus* breeding facilities contributes to the incidental release of this species. A tightening of rules in the regulation of aquaculture activities and the use of control programs for naturalized populations are necessary to address the increasing dispersion of the species in Argentina and potential ecological problems.

Keywords. feeding behavior, grasslands, naturalized populations, reproduction, invasive frogs.

Introduction

The American bullfrog, *Lithobates catesbeianus,* is native to southeastern and eastern Canada, east of the United States and northeast of Mexico (Frost, 2007). *Lithobates catesbeianus* has also been reported in another 41 countries throughout the world (Ficetola *et al.,* 2007a; Laufer *et al.,* 2008), where it was introduced for commercial purposes.

The presence of *Lithobates catesbeianus* outside of its native range negatively affects autochthonous communities through resource competition, direct predation, and breeding interference (Werner *et al.,* 1995; Kupferberg, 1997; Kiesecker and Blaustein, 1998; Adams, 2000; Kiesecker *et al.,* 2001; Blaustein and Kiesecker, 2002; Kats and Ferrer, 2003; Pearl *et al.,* 2004; Pearl *et al.,* 2005; Laufer *et al.,* 2008). Lowe *et al.* (2000) considered *L. catesbeianus* to be one of the most harmful invasive species of the world. Moreover, negative effects are further increased because bullfrogs can act as vectors of pathogens, affecting native amphibian populations all over the world (Berger *et al.,* 1998; Daszak *et al.,* 1999; Mazzoni *et al.,* 2003; Daszak *et al.,* 2004; Hanselmann *et al.,* 2004; Garner *et al.,* 2005; Garner *et al.,* 2006). Human-induced habitat alterations can also influence food-resource distribution, favoring exotic species over natives (Kiesecker *et al.,* 2001).

In southern South America, *Lithobates catesbeianus* has been introduced in Argentina, Brazil, and Uruguay (Borges-Martins *et al.,* 2002; Sanabria *et al.,* 2005; Laufer *et al.,* 2008); however, only one study has been published documenting negative effects on native vertebrate populations (Laufer *et al.,* 2008). Lavilla (2001) suggested that specimens of *L. catesbeianus* had escaped from a breeding facility in the province of Entre Ríos, Argentina, and could have established feral populations, alerting to their potential negative effects on native species (*i.e.,* competition, predation, potential dissemination of pathogens, etc). Sanabria *et al.* (2005) first recorded the American bullfrog occupying natural environments in San Juan province, Argentina, and discussed causes and possible consequences of its introduction. Recently, Pereyra *et al.* (2006) reported on the presence of *L. catesbeianus* in pristine and rural environments at several localities from the interior Atlantic Forest of

eastern Misiones province, the most amphibian species-rich area in Argentina.

Between 1989 and 1991, an American bullfrog breeding facility was developed in the locality of 9 de Julio, 9 de Julio district, Buenos Aires province, which was located along the RN 5 (national highway) (35°29'56.94"S 60°54'37.14"W) (Figure 1). The district was affected by abundant rainfall; rainfall between 415-600 mm was recorded in March 2001 and later that year 80% (about 340,000 has) of the district was either flooded or without dry ground (Ventimiglia *et al.,* 2003). According to Baloriani (pers. comm.), the presence of *Lithobates catesbeianus* was obvious during this period, and individuals were commonly seen in swimming pools in town and in flooded rural roads.

Herein, we report on an established population of *Lithobates catesbeianus* in Buenos Aires province, Argentina, along with data on stomach contents and reproduction at the study area. Considering that *L. catesbeianus* can act as a vector of *Batrachochytrium dendrobatidis* (Daszak *et al.,* 2004; Garner *et al.,* 2006), we assessed the presence of chytrid infections in this population.

Material and Methods

The study area is located in 9 de Julio district, province of Buenos Aires, about 250 km southwest from the city of Buenos Aires. The climate is humid, temperate, and the local landscape consists of grasslands with permanent lagoons. The area belongs to the phytogeographic zone of Distrito Pampeano Occidental (Cabrera, 1994), an area that has been highly exploited over the last 250 years.

Four surveys were conducted in 9 de Julio district, during the following periods: 26-28 December 2006, 13-15 January, 2007, 3-5 February, 2007, and 26-28 February, 2007. Three different environments were surveyed: 1) a seminatural permanent pond next to town (35°29'12.90"S 60°54'0.41"W), 2) a ditch in the town (35°27'12.10"S 60°51'57.20"W), and 3) a natural permanent pool in an adjacent rural zone (35°30'10.07"S 60°57'17.60"W) (Figure 1). Geographic coordinates were taken with a Garmin Map60 GPS. Each environment was sampled once per period, and the abundance was calculated with sampling effort of number of individuals collected by

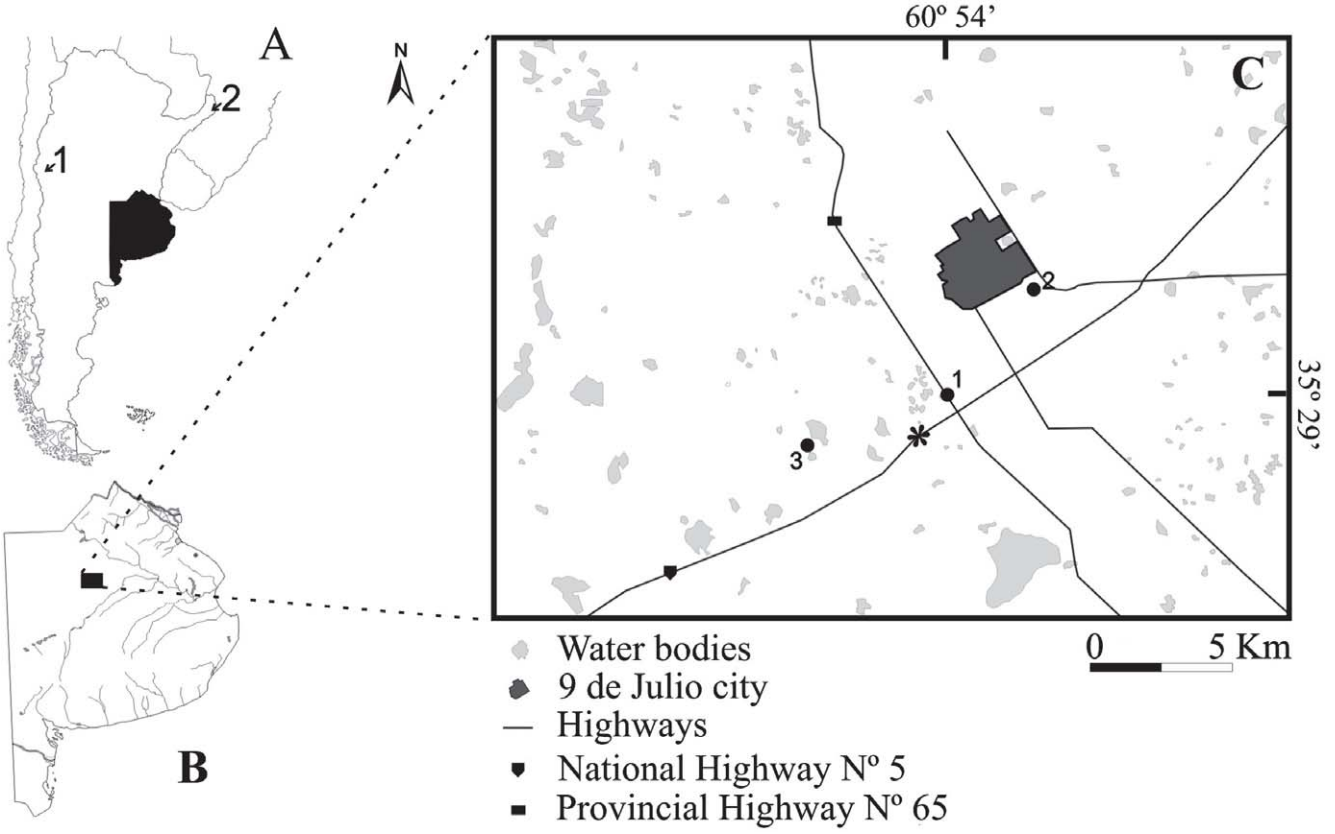

Figure 1. Site map. (A) Distribution of *Lithobates catesbeianus* in (1) San Juan province and (2) Misiones; (B) Buenos Aires province; (C) 9 de Julio Study area, showing: (*) abandoned breeding facilities, (1) permanent pond next to the town, (2) a ditch in the town, and (3) permanent pool in adjacent rural area.

three persons per day. Diurnal captures were made by angling and night captures were made by active searches (mainly of vocalizing males). Immediately after capture specimens were euthanized using an overdose of benzocaine, then fixed in 10% formaldehyde, and sexed using secondary sexual characters and/or direct observation of the gonads by dissection. Stomach contents were analyzed by removing the complete alimentary canal, as recommended by Schoener (1989). Prey items were determined using standard keys (Ringuelet *et al.,* 1967; Coronado-Padilla and Marquez-Delgado, 1978; Brewer and Arguello, 1980; Cei, 1980). A total of 35 specimens were collected, 16 were deposited in the Herpetological Collection at Museo de La Plata (catalogue numbers MLP-A 4949-4964).

We analyzed histological sections of 25 specimens. From each specimen, six skin sections (about 13.5 mm in size) were collected. Skin samples were removed from the ventral abdominal and pelvic regions of juveniles and adults, fixed in 10% formaldehyde, paraffin-embedded, sectioned at 5 µm, and stained with hematoxylin and eosin to diagnose the presence of *Batrachochytrium dendrobatidis* (*Bd*) with the aid of a bright field microscope, following Berger *et al.* (1999) and Herrera *et al.* (2005).

Results

The number of individuals of *Lithobates catesbeianus* collected were 13 females, 12 males, and ten juveniles. The relative abundance of bullfrogs in each environment (Table 1) in descending order was: Site 2 (n = 23), Site 1 (n = 11), and Site 3 (n = 1). *Rhinella arenarum, Leptodactylus ocellatus, Pseudis minutus, Hypsiboas pulchellus,* and *Pseudopaludicola falcipes,* were also observed at the study sites, both visually and acoustically.

Analysis of stomach contents showed that 85.7% (n = 30) had recently feed and a total of 11 different prey items were identified (Table 2). The diet of *Litho-*

Table 1. Abundance of females (F), males (M), and juveniles (J) of *Lithobates catesbeianus* collected in the three environments surveyed.

Period	Site 1			Site 2			Site 3		
	F	M	J	F	M	J	F	M	J
26-28/11/2006	4	3	1	1	2	2	0	0	0
13-15/01/2007	0	1	1	3	3	1	0	0	1
3-5/02/2007	1	0	0	3	2	2	0	0	0
26-28/02/2007	0	0	0	1	1	2	0	0	0
Total	5	4	2	8	8	7	0	0	1
Total per Site	11			23			1		

Table 2. Diet composition of females (F), males (M), and juveniles (J) of *Lithobates catesbeianus* examined at the three sites surveyed.

Prey ítems	Site 1			Site 2			Site 3	Total
	F N = 5	M N = 4	J N = 2	F N = 8	M N = 8	J N = 7	J N = 1	
INSECTA								
Coleoptera	21	2		8	10	7		48
Hemiptera (Heteroptera)				2	1			3
Hemiptera (Auchenorryncha)						1		1
Hymenoptera						5		5
Diptera						1		1
Orthoptera				1				1
CRUSTACEA								
Isopoda		2			4	3		9
ANNELIDA								
Oligochaeta		1						1
VERTEBRATES								
Pisces								
Cnesterodon sp.				5				5
Anura (tadpoles)								
Leptodactylus ocellatus				4				4
Lithobates catesbeianus		1		1				2
Plant material	3	2	1	5	3	2		16
Empty stomachs	1	0	1	0	2	0	1	5
Aquatic prey	0	3	0	13	5	1	0	22
Total prey	21	6	0	21	15	17	0	80

bates catesbeianus consisted of coleopterans (50%), vertebrates (11%) and isopods (9%). Coleopterans were the most frequently represented prey in 29 individuals. The second most frequent prey were isopods, found in 8 individuals. Plant material accidentally consumed was an important component representing 17% and found in 9 individuals. Coleopterans were the most abundant prey at site 1 (70%) and site 2 (43%). No prey items were found in the single specimen collected at site 3. Coleopterans were found in 58% of the females, 46% of the males, and 47% of the juveniles. The most frequently found prey in females was coleopterans and vertebrates, found in 12 and 7 individuals respectively. Coleopterans were also the most frequently found prey item in males and juveniles, found in 10 and 7 individuals respectively. Isopods were the second most frequent prey in males and juveniles, found in 3 and 5 individuals respectively. Aquatic prey were more commonly found in adults (29%) than juveniles (6%).

Acoustic activity of males of *Lithobates catesbeianus* was occasionally heard at the three sites surveyed. That said, we did not observe amplectic pairs nor did we collect tadpoles in our study sites. Reproduction is suggested by the presence of gravid females ($n = 3$), juveniles, and the recently eaten tadpoles inside stomachs of one of the collected specimens at site 1 and 2.

Stained skin sections from 25 individuals (6 juveniles and 19 adults) were examined for the presence of *Batrachochytrium dendrobatidis* or lesions consistent with chytridiomycosis. None of the analyzed tissues were infected and individuals did not show skin injuries commonly associated with chytridiomycosis.

Discussion

The installation of *Lithobates catesbeianus* breeding facilities contribute to the incidental release of this species and this practice is considered one of the primary sources for the introduction of bullfrogs in different countries throughout the world (Kupferberg, 1997; Daza-Vaca and Castro-Herrera, 1999; Casas-Andreu *et al.,* 2001; Lavilla, 2001; Diaz de Pascual and Chacón-Ortiz, 2002; Hanselmann *et al.,* 2004). The increasing demand of bullfrog meat as a source of food has intensified the development of breeding facilities in Argentina and Latin America (Mazzoni *et al.,* 2003). Data on the actual demand and profitability of this activity are not available for Argentina.

Ecological niche-modeling to assess the possible risk of invasions of *Lithobates catesbeianus* at a global scale were provided by Ficetola *et al.* (2007a), and at a smaller scale by Ficetola *et al.,* (2007b) for Europe and Giovanelli *et al.* (2007) for Brazil. These studies suggest that the environmental variables that most influenced the predictions were mean diurnal range temperature, annual mean temperature, precipitation of the driest quarter, and winter precipitation. Our report on naturalized populations of *L. catesbeianus* in the province of Buenos Aires, an area that models have predicted as being of very high suitability for American bullfrogs (Ficetola *et al.,* 2007a), indicates that all conditions are available for the proliferation of these invasive frogs.

The general trophic spectrum reported for *Lithobates catesbeianus* diets (Werner *et al.,* 1995; Daza-Vaca and Castro-Herrera, 1999; Hirai, 2004) is consistent with results of the present study (Table 1). However, as reported in other studies, *L. catesbeianus* can also prey on larger animals such as birds (Gollop, 1978; Wu *et al.,* 2005), turtles (Corse and Metter, 1980; Graham, 1984), snakes (Minton, 1949), bats (Lee, 1969; Kirkpatric, 1982), and weasels (Beringer and Johnson, 1995). Several studies demonstrate that aquatic prey and frogs constitute a substantial portion of the bullfrog diet (Korschgen and Moyle, 1976; Corse and Metter, 1980; Werner *et al.,* 1995; Hirai, 2004; Wu *et al.,* 2005). We observed similar diet characteristics in adult bullfrogs from 9 de Julio district. Aquatic prey items were more commonly found in adults (33%) than juveniles (6%); adults also preyed on tadpoles of *Leptodactylus ocellatus* and conspecifics. A large proportion of frogs and aquatic prey items in adults was also documented by Hirai, (2004); however, no differences were found by Werner *et al.* (1995). The difference in the proportion of aquatic prey between adults and juveniles showed in this study might be due to a difference in food microhabitat partitioning.

The introduction and dissemination of American bullfrogs in Argentina is an increasing conservation problem. *Lithobates catesbeianus* proliferated and dispersed successfully in natural and modified environments in Misiones province (Pereyra *et al.,* 2006). Also, this species is having a great impact on populations of *Rhinella arenarum* and *Leptodactylus ocellatus* in San Juan province (Sanabria *et al.,* 2005). Our data show that the species preys on tadpoles of *L. ocellatus* in Buenos Aires province, suggesting potential negative effects on native *L. ocellatus;* not only regarding larval predation but also adult competition for food. *Leptodactylus ocellatus* is a generalist predator, frequently feeding on aquatic and terrestrial

prey such as insects, crustaceans, annelids, mollusks, and vertebrates (frogs and fishes) (Gallardo, 1964; Basso; 1990; Maneyro *et al.,* 2004), overall a similar diet to that of the invasive bullfrog. In addition, the relative abundance of bullfrogs in 9 de Julio district shows a greater number of specimens at site 2, the most modified environment studied. This plasticity to occupy modified environments provides to the invasive *L. catesbeianus* an advantage as a strong competitor to native species. This is evidenced by the presence and proliferation of bullfrogs in three different environments in Argentina: the Interior Atlantic Rainforest in Misiones, the pre-Andean areas of San Juan, and the grasslands of Buenos Aires province. *Lithobates catesbeianus* represents a risk to native communities not only because of its presence but also as a function of its abundance.

Several studies worldwide have documented the presence of chytridyomicosis in *Lithobates catesbeianus* (Hanselmann, 2004; Garner *et al.,* 2005; Ouellet *et al.,* 2005; Garner *et al.,* 2006). The disease has been reported in native species of South America, including *Leptodactylus ocellatus,* from Buenos Aires province Argentina (Bonaccorso *et al.,* 2003; Seimon *et al.,* 2005; Toledo *et al.,* 2006; Barrionuevo *et al.,* 2008; Herrera *et al.,* 2005). Several authors have considered *L. catesbeianus* as a vector for chytridiomicosis. We found no evidence of *Bd* fungus infection in specimens of *L. catesbeianus* from 9 de Julio district. The absence of *Bd* infection in *L. catesbeianus* was also reported for populations in Brazil, Canada, France, Italy, and Japan (Garner *et al.,* 2006; Toledo *et al.,* 2006). Although the histological technique used can provide false-negative results, the technique has been previously used and it provides quick results at a low cost (Smith, 2007; Kriger *et al.,* 2007). In order to counteract the impact of potential false negative results, we analyzed a large number of cuts (n = 150). Studies aiming to monitor the presence/absence of *Bd* fungus in naturalized populations of American bullfrogs and native amphibians are needed to assess the extent of this epidemic in the region. Moreover, studies focusing on the interactions between *L. catesbeianus* and native communities are necessary.

A revision of the current legislation and regulations for captive breeding programs and the implementation of control protocols for naturalized populations are especially necessary to avoid the increasing dispersion of the species *L. catesbeianus* in Argentina and the potential ecological problems and impact it may have on natural environments.

Resumen

Numerosos estudios indican que la introducción de la "rana toro americana", *Lithobates catesbeianus,* produce efectos negativos sobre las comunidades nativas. En la presente contribución la "rana toro americana" es reportada por primera vez para la provincia de Buenos Aires, Argentina, en la localidad de 9 de Julio. Se examinó el contenido estomacal de los ejemplares colectados y la reproducción de esta especie fue confirmada en el área de estudio. Un total de 85.7% (n = 30) de los estómagos analizados presentaron contenido estomacal, identificándose un total de once ítems alimentarios. Estos ítems correspondieron principalmente a coleópteros (50%), y vertebrados (11%). Además, se evaluó la presencia del patógeno *Batrachochytrium dendrobatidis* en muestras de piel, pero el mismo se encontró ausente. La instalación de criaderos de *Lithobates catesbeianus* contribuye incidentalmente con la liberación de estos animales. Una mejora en la regulación de la actividad acuicultora es necesaria, como también lo es el empleo de programas de control de las poblaciones ya naturalizadas, para abordar la creciente dispersión de la especie en la Argentina y los problemas ecológicos que la misma implica en los ambientes naturales.

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