

## Climate change might drive species into reserves: a case study of the American bullfrog in the Atlantic Forest Biodiversity Hotspot

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**Habitat loss is the most important cause of species extinction worldwide that can act as a catalyst of biological invasions. To prevent species extinction, in-situ conservation strategies still figure as the cornerstone of biodiversity conservation. However, with expected broad-scale environmental changes likely to produce species range shifts and foster alien species invasions, will existing networks of protected areas be able to guarantee the long-term persistence of the species they should protect? Here we evaluate the potential impact of climate change in driving the invasive American bullfrog *Lithobates catesbeianus* into reserves currently established in the Atlantic Forest Biodiversity Hotspot. We produced potential geographic distribution maps both for current time and for 2050 using eight species distribution modeling methods and three different climate models to evaluate model uncertainty. We then overlaid a consensus prediction of species distribution to the existing network of protected areas. We show that, under climate change, *L. catesbeianus* is likely to colonize reserves more efficiently than today, putting in jeopardy native amphibian species thought to be protected in such places. We also suggest some policy recommendations to mitigate such potential impact on the Atlantic Forest native amphibian fauna.**

### INTRODUCTION

The designation and maintenance of protected areas is still the cornerstone of conservation strategies (LOUCKS et al., 2008). However, under projected broad-scale environmental

changes which will potentially shift the distribution of suitable habitats for many species across geographic space (PARMESAN, 2006; DINIZ-FILHO et al., 2009), concerns that existing networks of protected areas might not be able to guarantee the long-term persistence of the species they should protect have been expressed (PETERS & DARLING, 1985; SCOTT et al., 2002; ARAÚJO et al., 2004; HOLE et al., 2009). Up until now, little attention has been paid to the related problem that climate change might not only drive species out of reserves (see ARAÚJO et al., 2004) but might also foster the invasion of alien species into networks of protected areas (KLEINBAUER et al., 2009).

The process of alien species invasion is a key factor explaining local extinctions of native species due to the increased pressure that invasive species impose by changing local conditions in ecosystems, species assemblages and native populations (CATFORD et al., 2009). With the cascade of sensitive species extinctions caused by habitat fragmentation and loss (LAURANCE et al., 2002), new habitat conditions emerge with associated niches available to be occupied by species with high adaptability, such as invasive species that prevent recolonization by endemic species (KAISER, 1997; CATFORD et al., 2009). Usually, the introduction of alien species occurs through a human colonization process, which introduces species either intentionally (for hunting, pest control, food, among others) or unintentionally.

Alien species invasion would be especially dangerous in places where the existing network of protected areas is composed mainly of relatively small isolated forest remnants (KAISER et al., 1997). This is the case of the Brazilian Atlantic Forest, one of the world's most species-rich, yet endangered and understudied ecosystems (CARNAVAL et al., 2009). The Atlantic Forest is also a top-ranked Biodiversity Hotspot (MYERS et al., 2000) harboring a large number of endemic frogs (MORELLATO & HADDAD, 2000). This biome is threatened by severe habitat destruction, and areas with the same overall deforestation rate can have very different degrees of natural riparian vegetation remaining, as well as varying connectivity with terrestrial environments (RODRIGUES et al., 2009).

The American bullfrog, *Lithobates catesbeianus* (Shaw, 1802), is one of the most harmful invasive species worldwide (LOWE et al., 2000). Native to eastern North America it was introduced into southern Europe, Asia and South America (NORI et al., 2011). In Brazil, introductions date from the 1930s in association with aquaculture (FONTANELLO & FERREIRA, 2007). The species is usually recorded in southern and southeastern sites in the Atlantic Forest (BORGES-MARTINS et al., 2002; DIXO & VERDADE, 2006). Furthermore, scientists called on the need to focus on this biome, aiming to prevent further invasions in Brazil (GIOVANELLI et al., 2008; NORI et al., 2011).

Here we evaluate the potential impact of climate change in driving the American bullfrog into reserves currently established in the Atlantic Forest. We produced several potential geographic distribution maps for this species and overlaid them to the existing network of protected areas. More importantly, we show that, under climate change, *L. catesbeianus* is likely to colonize reserves more efficiently than today, putting in jeopardy native amphibian species thought to be protected in such places.

## MATERIAL AND METHODS

We modeled the potential geographic distribution of the American bullfrog, an invasive species occurring in the Atlantic Forest Biodiversity Hotspot. The estimated current geographic range of the species was overlaid onto a grid with 468 cells with 0.5° of latitude and longitude. We obtained the range map of *L. catesbeianus* available at the IUCN Red List of Threatened Species™ (<<http://www.iucnredlist.org>>). The range was taken as a proxy for the species' Extent of Occurrence. We used this polygon map to generate a presence-absence matrix instead of point-locality data (for a similar approach, see LAWLER et al., 2009; DINIZ-FILHO et al., 2009a–b, 2010). An advantage of this is the possibility of working across a very large geographic space without needing to deal with sampling bias. However, polygon-based maps could overestimate species occurrence across space.

It is usual to model invasive species distributions based upon species' native range to identify suitable potential areas of invasion, i.e., realized climate space is modeled in one region and modelers ask whether the inferred climate envelope “transfers” to the realization of the fundamental climate space that has occurred elsewhere. However, studies that applied such methods have produced inconsistent records of transferability (transfer of climate associations from the native region to another prospective invasion range; see RODDA et al., 2011), especially in the case of the American bullfrog (GIOVANELLI et al., 2008) and cane toads (*Bufo marinus*; KEARNEY et al., 2008). For the cane toad, in particular, it has been determined that introduced ranges reflect a greater climatic range than those found within the native range of the species (KEARNEY et al., 2008). Since BEAUMONT et al. (2009), it has been reported that the impact of calibrating invasive species distribution models using native ranges will just misrepresent potential invasive distributions, and “these errors will propagate when estimating climate change impacts” (BEAUMONT et al., 2009). Based on these studies, we decided to model *L. catesbeianus* potential distribution (areas at risk of invasion) based only on known invaded localities in Latin America, following RODDA et al. (2011).

There are several methods to model species' occurrences as a function of environmental variables (for a recent review and comparative evaluations, see ELITH et al., 2006). These methods range from simple bioclimatic envelope models, to complex artificial-intelligence based algorithms, and are based on different mathematical and statistical backgrounds, providing thus slightly different results (ELITH et al., 2006). As a consequence of the long controversy around the appropriate method to model species distribution, ARAÚJO & NEW (2007) proposed that a more reliable approach to predict species distributions should be based on a combination of methods, generating ensembles of forecasts (see DINIZ-FILHO et al., 2009).

Here we produced such ensembles of forecasts by modeling the species geographic distribution as a function of seven climatic variables (precipitation seasonality; temperature annual range; minimum temperature of coldest month; temperature of the colder and hottest months; precipitation of drier and wettest months) derived from three different coupled Ocean-Atmospheric Global Circulation Models (AOGCMs), for the A1 scenario for the year 2050: CCSM3, CSIRO and HADCM3. Climatic data were obtained from the WORDCLIM database (<<http://www.worldclim.org/>>) and all of the selected variables were converted to a

grid resolution of 0.0417 degrees (nearly 4 m). The WORDLCLIM database provides 19 bioclimatic variables; however, there is a large amount of collinearity among them, which could affect the efficiency of the model (HEIKKINEN et al., 2006). Thus, we selected climatic variables that are not collinear (the coefficients of correlation among selected variables were < 0.8) and climatic variables important to distribution and survival of amphibians species, mainly related with temperature and water availability (see GIOVANELLI et al., 2008).

We used eight different niche models to generate the extents of occurrence for the species: a simple surface range envelope model based on orthogonal limits (BIOCLIM); Euclidian Distances; Mahalanobis Distances; Gower Distances (or Domain); General Linear Model (GLM); Genetic Algorithm for Rule Set Production (GARP); Random Forest and Maximum Entropy (MAXENT). These methods have been widely used and comparative analyses of their statistical performance can be found elsewhere (ALLOUCHE et al., 2006; ELITH et al., 2006; TSOAR et al., 2007; PHILIPS & DUDÍK, 2008).

We obtained models as follows. First, for each of the eight niche models, we randomly partitioned the species occurrence into two subsets: 70 % for calibration, 30 % for validation, and this procedure was replicated 50 times. Then, we truncated each model based on the receiver operating characteristic (ROC) curve (see ALLOUCHE et al., 2006), transforming quantitative predictions of some models (e.g., distances in Mahalanobis or environmental suitability from MAXENT) into a binary vector of 0/1, indicating absence or presence of the species in each grid cell. For each model, thresholds were determined using the ROC curve by applying the maximum sensitivity (true positive rate) for a minimum of 1 – specificity value (false positive rate). For building the ROC curve, we obtained pseudo-absences using the cells in the Atlantic Forest outside the species' extent of occurrence. After doing this, we expressed the occurrence of the species by the frequency at which it appears in a given cell.

Finally, following ALLOUCHE et al. (2006), we evaluated the performance of the models by the True Skill Statistics (TSS), given by:  $TSS = sensitivity + specificity - 1$ ; where sensitivity is the proportion of correctly identified presences and specificity is the proportion of correctly identified absences, both in the validation dataset. There is no consensus over the validity of statistical measures of fit and transferability (see PETERSON et al., 2007), but we followed the approach proposed by ARAÚJO & NEW (2007) and focused on the results provided by an ensemble solution derived from the distinct models and, in this way, avoided discussions about their relative performance. We based our final analyses on a total of 1200 projections (400 models projected into three AOGCMs). We overlaid each of the predictions and produced an unweighted consensus of the frequencies. Weighting predicted occurrences by the average TSS of models did not qualitatively affect our results (especially because we excluded poor models, with low TSS statistics, before calculating predictions). We implemented all niche models in the new integrated computational platform “Bioensembles”, (see also DINIZ-FILHO et al., 2009).

To evaluate the potential impact of the American bullfrog on the existing network of protected areas established in the Atlantic Forest, we considered 21 grid cells to be already protected in the biome, because they encompass protected areas in I-IV IUCN categories with more than 5000 hectares, and overlapping with at least 25 % of total grid cell area. Then we overlaid the ensemble solutions produced for current time and for 2050 to evaluate the spatial overlap between *L. catesbeianus*, current and future distribution with existing protec-

ted areas. To test if the species is more likely to colonize existing reserves in the future than now, we calculated the mean consensus value (i.e., mean frequency at which the species appears in a given cell according to each model) for the 21 grid cells composing the existing network of protected areas. Then, we drew 1000 random sets of 21 grid cells and calculated the mean consensus value for each of these random samples. After building a randomized frequency distribution of these values, we assessed the probability of the observed consensus values being equal to or lower than those obtained in random samples. Tests were done both for current time and for 2050.

## RESULTS

The fit statistics of the models, for each AOGCM vary, but we found relatively high TSS values around 0.7, with values around 75 % or higher of the presences being correctly identified by all models – thus, showing the accuracy of our models. The consensus maps obtained for the current time match the occurrence of *L. catesbeianus* throughout the Atlantic Forest, from southern regions up to the northeast coast of Brazil, but relatively high frequencies ( $> 0.5$ ) appear mainly for the mid-central Atlantic Forest (fig. 1A). There were also clear changes of *L. catesbeianus* distribution in 2050, characterized by a geographic range reduction and a mid-south shift of the higher mean consensus value, and a decrease in predictions in the northeast region (fig. 1B).

The maps showing the standard deviation relative to the consensus mean value (i.e., the uncertainty associated to the use of different niche models and AOGCMs) indicate that the largest differences among maps appear in the western region for the Atlantic Forest (for models derived for current time, fig. 1C), and in the mid-south and east coast of the biome (for models built for 2050, fig. 1D). Lower uncertainties were found for the northeast of the Atlantic Forest (fig. 1C–D).

The existing network of protected areas concentrates reserves in the eastern region of the Atlantic Forest, with some protected areas located in the western and northern regions (fig. 2). Both for current time and for 2050, sites with relatively high values of mean consensus prediction ( $> 0.5$ ) overlap these protected areas (see tab. 1 for reserve names). Although mean consensus values decrease in the future, due to a range contraction of *L. catesbeianus* (fig. 3), overlap of species occurrence with existing reserves in the future was higher than expected by chance (Prandomization  $< 0.05$ ; fig. 3B), when compared to such overlap in current time (Prandomization  $> 0.05$ ; fig. 3A).

## DISCUSSION

Our results indicate that climate change is likely to induce a geographic range contraction of the American bullfrog, but worryingly this might also drive this alien species into protected areas currently established in the Atlantic Forest. Invasion risk will grow at a higher rate than expected considering the present distribution of the species, pointing to a potential bias in

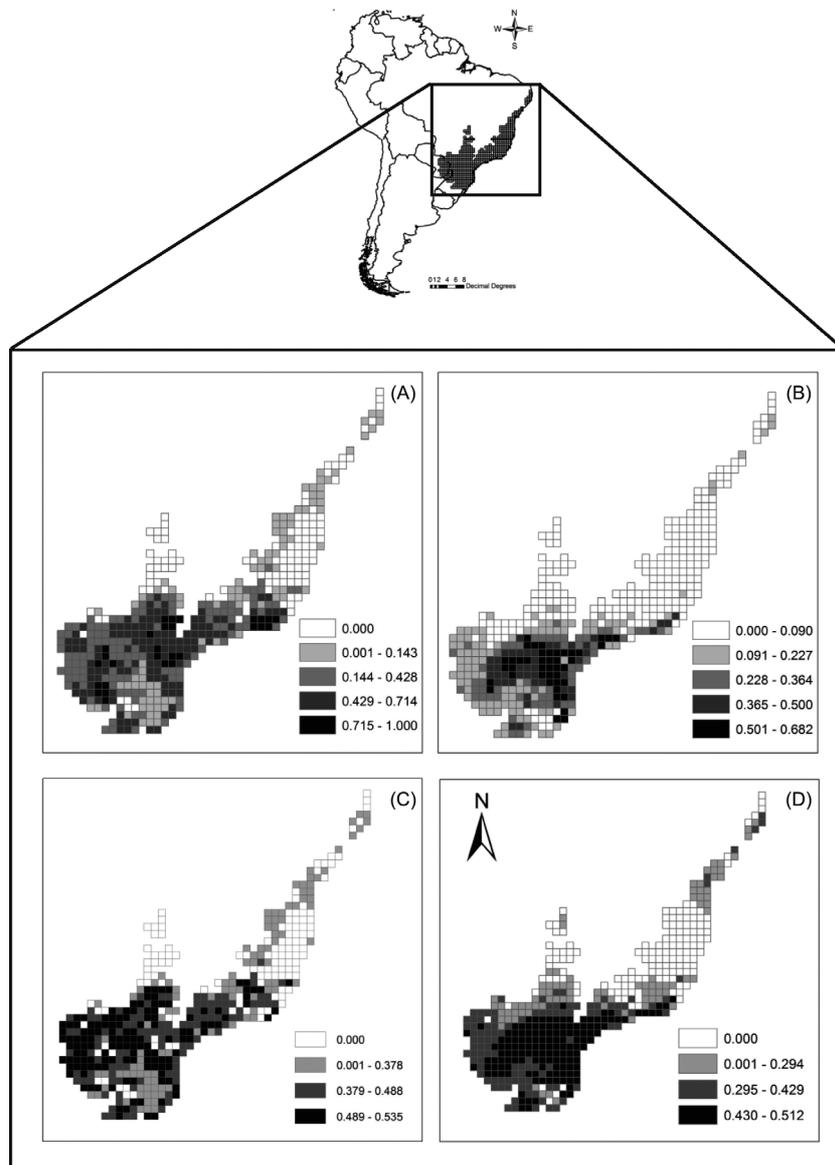


Fig. 1. – Maps of geographic range predicted by the consensus in ensembles of forecasts (mean consensus value, see text for further details) of the American bullfrog *Lithobates catesbeianus* distribution in the Atlantic Forest Biodiversity Hotspot for (A) current time and (B) 2050 on a grid with 469 cells of 0.5° of latitude and longitude covering the biome; (C) shows spatial patterns of model uncertainty associated to predictions for (C) current time and (D) 2050.

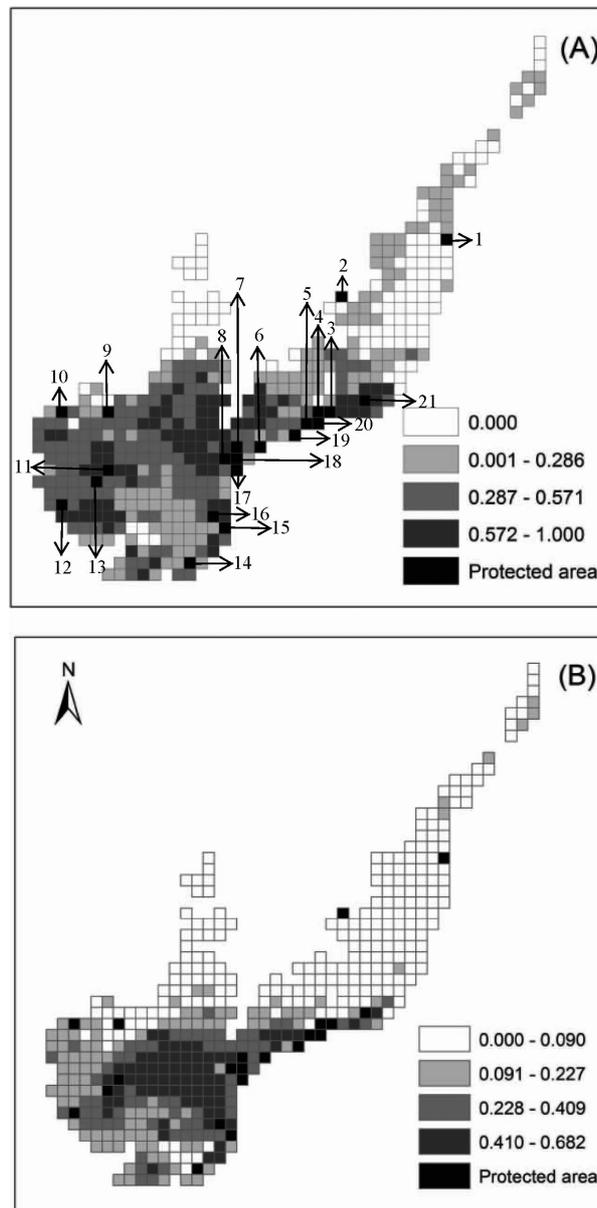


Fig. 2. – Spatial overlap between the existing network of protected areas and geographic range predicted by the consensus in ensembles of forecasts of the American bullfrog *Lithobates catesbeianus* distribution in the Atlantic Forest Biodiversity Hotspot for (A) current time and (B) 2050.

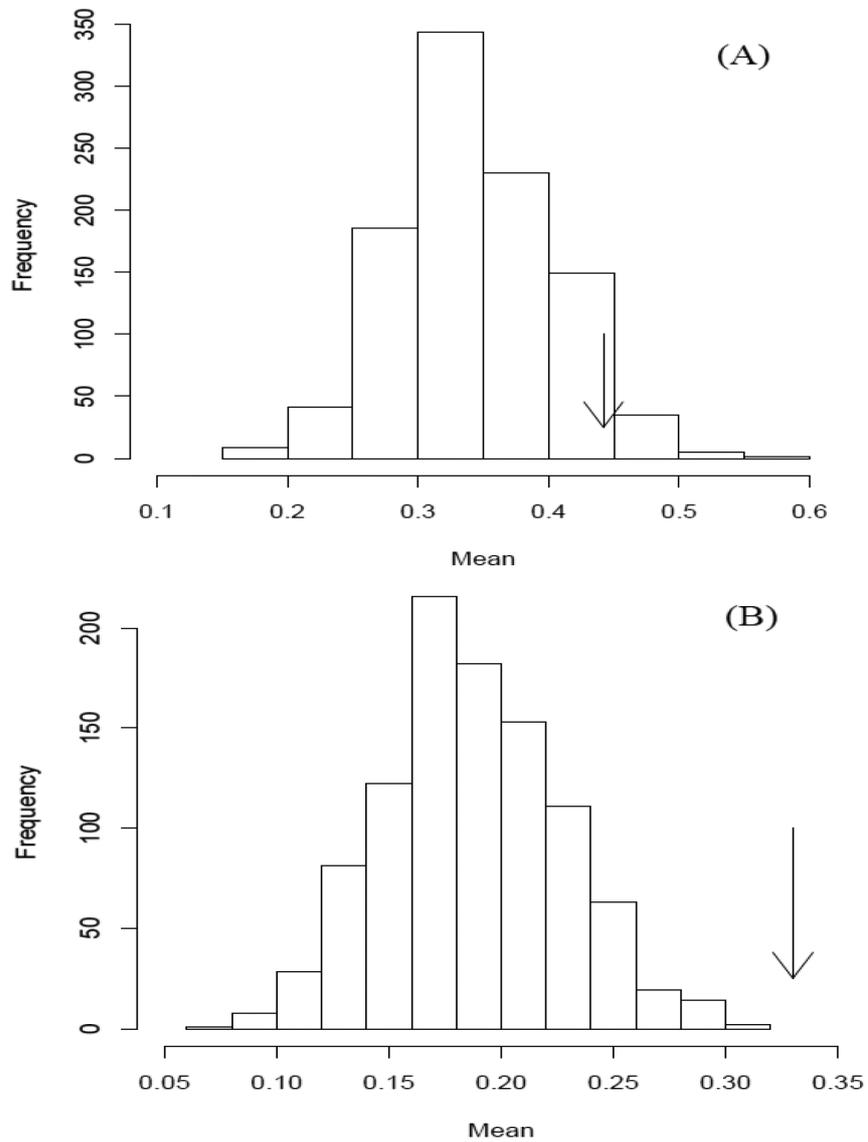


Fig. 3. – Distribution of mean consensus values based on ensembles of forecasts of the American bullfrog *Lithobates catesbeianus* distribution in the Atlantic Forest Biodiversity Hotspot obtained by 1000 random sets resampled with 21 grid cells for (A) current time and (B) 2050. Arrows indicate the observed mean values (i.e., those found for the 21 currently protected grid cells) for each time frame.

Table 1. – Grid cells indicated in figure 2A that harbor protected areas I-IV IUCN Categories (being also > 5000 ha) and their respective names.

Grid cell	Protected area
1	Reserva Biológica de Uma
2	Parque Nacional das Sempre-Vivas
3	Parque Estadual do Cunhambebe Estação Ecológica de Bananal
4	Parque Nacional da Serra da Bocaina
5	Parque Estadual Serra do Mar
6	Parque Estadual Serra do Mar Estação Ecológica Juréia-Itatins Parque Estadual Prelado Parque Estadual Itinguçú
7	Parque Estadual Carlos Botelho Parque Estadual Intervalles
8	Parque Estadual do Alto Ribeira Parque Estadual Caverna do Diabo Parque Estadual das Lauráceas
9	Parque Estadual Várzeas do Rio Ivinhema
10	Parque Nacional Cerro Corb
11	Parque Nacional do Iguaçú
12	Reserva Biológica São Rafael
13	Parque Nacional do Iguaçú Parque Provincial Uruguay
14	Parque Nacional de Aparados da Serra Parque Nacional da Serra do Geral
15	Parque Estadual da Serra do Tabuleiro
16	Reserva Biológica da Canela Preta Parque Nacional da Serra do Itajaí
17	Parque Nacional do Superagui Estação Ecológica de Guaraqueçaba Parque Estadual do Rio Turvo
18	Parque Estadual do Rio Turvo Parque Estadual Caverna do Diabo
19	Parque Estadual Serra do Mar
20	Parque Nacional da Serra da Bocaina Parque Estadual de Parati-Mirim Parque Estadual Serra do Mar Estação Ecológica de Tamóios
21	Parque Estadual dos Três Picos Estação Ecológica do Paraíso

legal protection (mainly driven by reserve establishment based on political decisions rather than scientific ones) towards high mountain areas and sites with low agricultural potential (LOYOLA et al., 2008), especially in the mid-south region of the biome (the Brazilian state of São Paulo; see BECKER et al., 2010). This assumes even greater importance, because when we consider the current extent of occurrence of this species in the Atlantic Forest (i.e., the range map provided by IUCN, without modeling the species distribution – which is used in most conservation assessments), there is a low spatial overlap between the species' range and protected areas – only 2 out of 21 cells are supposed to be already occupied by *L. catesbeianus*.

It is worth noting that, as ecosystems are fragmented and degraded inside (like the Atlantic Forest), native species increase their stress reducing their resilience to invasions (CATFORD et al., 2009). Thus, the presence and persistence of invasive species becomes strong in natural environments, generating the extinction of native species that generally have spatial and ecophysiological requirements (e.g., KAISER, 1997). The extinction of specialized functional groups impoverish the native species ensemble and its ecological functions, generating the loss of ecosystem services of provision (e.g., timber), regulation (e.g., floods, warm climate) and support (e.g., pest control of disease vectors and for food production, crop pollination, seed dispersal), with fatal consequences for the economy and human health.

Even worse, there is evidence that climate change may trigger the infection of amphibians by fungal pathogens (POUNDS et al., 2006), in particular the fungus *Batrachochytrium dendrobatidis* (an invasive organism recently introduced into the Americas and Australia; see POUNDS et al., 2006) as temperatures at many highland localities are shifting towards the growth optimum of *B. dendrobatidis*, thus encouraging disease outbreaks (POUNDS et al., 2006) – although the relationship between *B. dendrobatidis* and climate change seems to be still controversial (CAREY & ALEXANDER, 2003; LIPS et al., 2008). Despite controversy, it is clear that climate change could affect biodiversity not only by shifting species' geographical ranges, but also by affecting complex interactions taking place at the local and regional scales, including the enhanced risk of alien species invasion. Hence, native amphibian species inhabiting reserves that compose the existing Atlantic Forest network of protected areas might not be secure, being affected by alien species invasion, climate change and fungal pathogens (ETEROVICK et al., 2005).

Beyond potential impacts of *L. catesbeianus* invasion in the biome are the caveats imposed by the ensemble forecasting approach (ARAÚJO & NEW, 2007). Species distribution models derived from distinct modeling techniques and climate models (AOGCMs) entail different predictions about the species occurrences, which were evidenced by uncertainty maps (fig. 1C–D). We should bear in mind that our modeling approach is based on modeling species' distribution within the Atlantic Forest and does not take into account the fact that the distribution (size, shape and location) of the biome itself can change (SALAZAR et al., 2007). Climate change may also shift the geographic distribution of species present in surrounding biomes, which were not included in this analysis, and could enter the new limits of the Atlantic Forest. Potential shifts in the ranges of these species may affect our forecasts by enriching or impoverishing the Atlantic Forest's ecotones or altering community composition at the edges of the biome, and thus their resistance to species invasion. Further, we modeled only the distribution of one amphibian species, and climate change will induce range shifts in many others. Even with these potential drawbacks, we rely on the fact that the occurrence of

*L. catesbeianus* in areas of the Brazilian Atlantic Forest is especially concerning, and that it is likely that this particular species has deleterious effects on populations of native amphibians and other organisms through competition and predation (see GLOWE et al., 2000; GIOVANELLI et al., 2008; NORI et al., 2011).

### POLICY RECOMMENDATIONS

It is essential that the government makes an additional effort to collaborate with universities, research institutes, governmental and non-governmental environment agencies, regional and national environmental corporations, to conduct continuous monitoring of native biodiversity. They should also strive to set up an early detection system of invaded ecosystems to make following and control of invasive species easier, as well as facilitating the granting of permits for hunting or control (e.g., for domestic consumption without captive live organisms) for local people and generating a list of prohibited species for husbandry activities and introduction into jurisdictions, to prevent the invasion of new habitats (HELLMAN et al., 2008).

Governmental authorities must keep in mind that captive animals maintained outside their range can escape from captivity and become pests or invasive species causing tremendous damage to agriculture and fisheries, but also to native animal populations (PIMENTEL et al., 2005; GIOVANELLI et al., 2008). This is exactly the case of the American bullfrog in the Atlantic Forest. This paper aimed to provide basic information on current and future potential distribution of this invasive species and to call attention to invasion risk in protected areas, as well as identifying those regions that need to be prioritized for monitoring herpetological invasions.

Current and new legal and normative frameworks must promote harmony of legal instruments and cooperation among different sectors and within different government levels to control the spread and eradicate populations of the American bullfrog in Brazil (GIOVANELLI et al., 2008; NORI et al., 2011). We believe it is crucial to review and articulate environmental and economic development policies in the light of the problems related to invasive species. At the same time, Brazilian regional development policies must incorporate the issue of invasive species and take into account measures to prevent their invasion and dispersal.

Scientific studies on *L. catesbeianus* ecology and distribution should seek to contribute to building technical, human and institutional capacities in order to strengthen decision makers (e.g., government staff) and local stakeholders in terms of implementing actions related with the invasion of this particular species (see GIOVANELLI et al., 2008). Hence, it is necessary to design and establish governmental protocols for eradication, control and management of the American bullfrog populations. Governments also need to encourage, through economic incentives, the participation of a well informed citizenship.

From an economical point of view, it is critical to nationally establish mechanisms to obtain, exchange, manage and access information on the American bullfrog for a better understanding of the likely consequences of its biological invasions, as well as how to mitigate

its ecological, social and economic impacts on invaded ecosystems. Such knowledge must support governmental decision-making and improve communication, education and awareness of the Brazilian civil society, so as to obtain additional support and participation on *L. catesbeianus*' control and eradication.

Policies on national economic development must provide ways to prevent *L. catesbeianus* entry and dispersal within the country; and to decrease commercialization of this species by stimulating the substitution of its trade, by one based on native species or even based on exotic species of lower risk of invasion. The legal framework and guidelines for the handling of the American bullfrog in the country must be reinforced, including biosafety procedures on *B. dendrobatidis* infection and strict control for the importation, use or translocation of individuals (eggs, tadpoles and adults) within the country.

Finally, predictive distribution models for the natural dispersal of the populations of this species must be a research priority to adequately inform local risk management and periodically generate early warnings. Along with this, a hard research line is still needed to answer some management-related questions such as: (1) which other factors besides climate change can cause the reduction of the geographical spread of an invasive species?; (2) how do species invasions interact with habitat fragmentation in the Atlantic Forest?; (3) what kind of biotic interactions and mechanisms exclude invasive species at local scale?; (4) which is the sequence of local extinctions of both species and functional groups once their habitat is invaded?

Answering these questions is certainly not easy and needs both scientific and political engagement. As highlighted by GIOVANELLI et al. (2008), "research is needed to start understanding the dynamics of American bullfrog populations in Brazil, while planning should focus on the eradication of invasive populations and aquaculture farming control". Production of bullfrog meat in aquaculture farms should also be strictly regulated and monitored by the Brazilian government to avoid further accidental introductions. We hope this paper contributes to a joint framework for the development of different strategies for biodiversity conservation, adding to burgeoning initiatives to plan the application of finite funds and efforts (including alien species management and control, establishment of protected areas, and ecosystem restoration) where they will be most effective.

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