# Buying environmental problems: the invasive potential of imported freshwater turtles in Argentina

Javier Nori[1,*], Geiziane Tessarolo[2], Gentile F. Ficetola[3], Rafael Loyola[4, 5], Valeria Di Cola[6], & Gerardo Leynaud[1]

**Authors' affiliations:**

[1]Centro de Zoología Aplicada and Instituto de Diversidad y Ecología Animal (IDEA-CONICET), Universidad Nacional de Córdoba, Rondeau 798, Argentina.

[2]Programa de Pós-graduação em Ecologia e Evolução. Universidade Federal de Goiás, Goiânia, Goiás, Brazil.

[3]Laboratoire d'Ecologie Alpine (LECA), Université Grenoble-Alpes. Grenoble 38000, France and LECA, CNRS, Grenoble 38000, France

[4]Laboratório de Biogeografia da Conservação, Departamento de Ecologia, Universidade Federal de Goiás, Goiânia, Goiás, Brazil.

[5]Brazilian Research Network on Climate Change – Rede Clima.

[6]Department of Ecology & Evolution (DEE) - University of Lausanne, Biophore Building, 1015 Lausanne, Switzerland.

*** Corresponding author's address:**

Javier Nori. Centro de Zoología Aplicada | Universidad Nacional de Córdoba. Rondeau 798, Córdoba, Córdoba |Argentina | Email: javiernori@gmail.com

## Abstract

Over the past years, decision-makers in Argentina have allowed the legal importation of thousands of specimens of freshwater turtles. Given their invasive potential, many of the imported species have been established and spread in other countries. The three most imported species in the last period have been *Graptemys pseudogeographica*, *Trachemys scripta*, and *Pseudemys nelsoni*, all of them native to North America. Here, the invasive potential of these species in Argentina was assessed based on (i) bioclimatic envelope models, (ii) distribution of water bodies, (iii) location of the most populated cities, (iv) comparisons between their alien and native climatic niches, and (v) the main ecological traits of those species. All these species were found to be able to establish viable populations in Argentina, especially *T. scripta* and *G. pseudogeographica*. This is because the country offers large amount of suitable climatic space for these species. Additionally, for both these species, these climatically suitable areas contain large areas with rivers and water bodies. The situation is especially problematic in freshwater ecosystems of the northeast, as well as in the most populated portion of the country. Multiple regulatory policies are suggested, which could avoid great ecological problems such as biodiversity loss and economic losses in the near future.



**Keywords:** alien species, conservation policy, niche overlap, species distribution modeling, *Trachemys scripta.*

## Introduction

Biological invasions are one of the major cause of species extinctions worldwide (Gurevitch and Padilla, 2004; Lowe *et al.*, 2004). Further, their impact on human activities generates multi-million monetary losses every year (Pimentel *et al.*, 2005; Charles and Dukes, 2007; Simberloff *et al.*, 2013). The establishment of several alien species is associated to food production and/or the development of regional economies (Pimentel *et al.*, 2005). However, considering amphibians and reptiles, recreational activities such as possession and pet trade are the most significant cause of establishment of alien species (Kraus, 2009). Amongst reptiles, freshwater turtles are probably the favorite species among pet-holders (Bush *et al.*, 2014). As a result, many of these species have established and dispersed outside their native range, generating multiple conservation problems in freshwater ecosystems (Cadi et al. 2004; Cadi and Joly, 2004; Ceballos and Fitzgerald, 2004).

Among the conservation problems associated with alien freshwater turtles (e.g. the Slider Turtle *Trachemys scripta*), significant increases in the mortality of native species which are competitively displaced by alien turtles have been shown in freshwater ecosystem (Lever, 2003; Cadi and Joly, 2004). Additionally, some species of freshwater turtles can hybridize with native related species, generating offspring with a low fitness due to the introgression of less adapted alleles and determining a strong genetic pollution of native genotypes, and the potential loss of native genotypes (Parham *et al.*, 2013; Figuereido, 2014). Another reported conservation problem is the disruption in the food-webs in aquatic ecosystems, associated with high population densities and generalist feeding habits of these alien species (Prévot-Julliard *et al.*, 2007; Kikillus *et al.*, 2010), or a negative effect in birdlife, induced for example by basking activities of alien species in water-bird nests (Lowe *et al.*, 2004).

When several introductions take place under suitable climatic conditions, the probability of success of a new biological invasion is large (Bomford *et al.*, 2009). Once established, invasive species generate complications with enormous ecological and economic costs, and eradication and habitat restoration becomes extremely difficult and expensive. Preventing the establishment is still the best and cheapest way to confront biological invasions (Simberloff *et al.*, 2013). In the last years, efficient methods and step-by-step approaches have been developed, allowing the assessment over broad geographical scales of the invasive potential and the ability of establishment associated with alien species and/or "potential invaders", on the basis of its climatic tolerances (Gallagher *et al.*, 2010; Broennimann *et al.*, 2012; Guisan *et al.*, 2014). A suitable use of these advances through the interaction between science and policy could prevent the above-mentioned problems.

Considering Amphibinas and Reptiles, in Argentina,, only one species has reached an important magnitude of invasion: the American Bullfrog (Akmentins and Cardozo, 2009; Nori *et al.*, 2011a). To date there are only a few isolated records of non-native species of reptiles, mainly associated to urban centers, which apparently have not yet established nor dispersed within 'natural areas' the country (e.g. *Hemidactylus mabouia* and *Tarentola mauritanica;* Baldo *et al.* 2008). Nevertheless, it is worrisome that one of the world's most invasive turtles, *T. scripta* (Lowe *et al.*, 2004), never found before in the country, has now been repeatedly recorded in the last three years (Alcalde *et al.*, 2012; Prado *et al.*, 2012; Quiroga *et al.*, 2015).

The situation in Argentina is now alarming, as every year authorities allow the legal importation and trade of thousands of individuals belonging to several species of freshwater turtles [Ministerio de ambiente y desarrollo sustentable de Argentina (Ministry of Environment of Argentina); Walter Prado, *pers. com*.], many of which hold a great invasive potential (Masin *et al.* 2014). To make things worse, nothing is known about the potential of these 'massively imported species' of establishing and generating ecological problems and biodiversity losses in the country. The most populated areas of the country, and especially those large cities which receive international importations, are highly vulnerable to the arrival (and potential further establishment) of alien species. Additionally, the country harbors large and very diverse freshwater ecosystems, such as the Argentinian Litoral, which contains the Parana river basin, one of the most important worldwide (Bonetto *et al.*, 1986; Olson *et al.*, 2001). Logically, these kind of ecosystems are also prone to be strongly affected by these potential new threats. Given this picture, studies on the distribution, trends and invasive risk of the most imported freshwater turtles in the national territory can inform and guide conservation policies.

The main goals of this study are to (1) determine the invasive potential of the most imported freshwater turtles in Argentina, (2) map places that are particularly vulnerable for species invasion, and (3) suggest regulatory policies for controlling this situation. By offering this information to decision-makers, it is possible to support stronger regulatory policies related to the possession, trade and breeding of imported turtles in Argentina.

## Materials and Methods

### *Species' records*

Species were selected based on the list of imported freshwater turtles in Argentina for the period 2000-2012, kindly provided by the Ministry of Environment of Argentina. All species with more than 1000 legally imported individuals in the country during the above-mentioned time frame were selected: *Graptemys pseudogeographica* (2600 individuals), *Pseudemys nelsoni* (1875 individuals) and *Trachemys scripta* (1185 individuals).

A database containing occurrence records from both the native (North America) and non-native geographical ranges (Worldwide) of these species was compiled and organized. Relevant literature was searched (e.g. Kikillus *et al.* 2010; Bugter *et al.* 2011; Masin *et al.* 2014) and some native records, from Global Biodiversity Information Facility (GBIF; www.gbif.org) were included. GBIF data have a certain degree of error, therefore only records derived from preserved specimens, and deposited at scientific institutions located in the country of origin of the species were considered (following Maldonado *et al.* 2015). In addition, records were considered as native if they fell within the distributional ranges of each species published by the International Union for the Conservation of Nature (IUCN, 2015). For non-native ranges, only records of populations for which the establishment has been explicitly confirmed were considered. The confirmation of the establishments was obtained from different sources [eg. Alarcos *et al.*, 2010;; Kikillus *et al.* 2010; Bugter *et al.* 2011; Rob Bugter *pers. com.*; Masin *et al.* 2014; unpublished information from Ravon Fundation (http://www.ravon.nl), United State Geological Survey (http://www.usgs.gov)]. The final database contained 446 individual records (222 records for *T. scripta*, 164 records of *G. pseudogeographica* and 60 records for *P. nelsoni*) (see Figure S1 and Appendix S1 in Supplementary Material).

***Climatic variables***

A Pearson's correlation was run between 19 bioclimatic variables at spatial resolution of 10 km retrieved from the WorldClim (www.worldclim.org; Hijmans *et al.* 2005) and five variables were selected based on their limited collinearity (<0.75). Selected variables were chosen considering natural history and physiological limits of the studied species (see Rödder *et al.* 2009). All the studied species strongly depend on the availability of water bodies for survival, therefore Annual Precipitation and Precipitation of Driest Quarter were included in the models variables determining availability of water for the species. Additionally, turtles are ectothermic, oviparous and mainly diurnal species. Therefore, reproduction, activity periods, feeding and survival all are strongly related to the availability of thermal energy.Therefore, the Annual Mean Temperature (in concordance with an energetic balance over the year, see Röder *et al.* 2009) and Mean Diurnal Range of temperatures were included as variables in the models. Finally, since freshwater turtles are also influenced by vegetation cover and topography with certain characteristics, the mean average vegetation fraction 2000-2012 (Broxton *et al.*, 2014) and the elevation (available at worldclim database) were included as additional variables.

***Bioclimatic envelope models (BEMs)***

Bioclimatic envelope models were built to estimate climatically suitable places for the selected species in Argentina. There are many alternative BEMs algorithms, with different levels of accuracy under different circumstances (Diniz-Filho *et al.*, 2009). A proposed solution to take into account this variability is to combine different algorithms into ensembles in order to find "areas of consensus" (as suggested by Araújo and New 2007). Here, ensembles of different modeling techniques in the R package (R Core Team, 2014) biomod2 (Thuiller *et al.*, 2014) were generated. The ensemble included six different modeling methods: Generalized Additive Models (GAMs), Classification Tree Analysis (CTA), Multiple Adaptive Regression Splines (MARS), Random Forest Models (RF), Generalized Linear Models (GLM) and Generalized Boosting Models (GBM). Models were calibrated using a subset of 70% of the records of each species, including records from both the native and invasive range of the species (as suggested by Gallagher *et al.* 2010). Pseudo-absences were defined for model calibration as twice the number of presences.

The definition of the model training region has important implications for model outcome and evaluation (Lobo *et al.*, 2008; Barve *et al.*, 2011), even for studies on invasive species (Nori *et al.*, 2011b; Rodda *et al.*, 2011), in which training areas must consider native but also invasive ranges (Gallagher *et al.*, 2010). Here, the training regions were defined as a buffer surrounding the presences in order to avoid (a) including pseudo-absences in potential suitable places for the species where it has not been introduced (see Rodda *et al.* 2011); and (b) generating pseudo absence in areas where the dispersal of the species is impossible (Godsoe, 2010). Given the importance of considering dispersal ability of the species in the definition of the training area (Barve *et al.*, 2011), the buffer area was defined using the maximum distance between a population and its nearest one for each species. This could be interpreted as an extreme measure of the ability of the species to colonize far places.

Models were projected into limits of Argentina to map climatically suitable areas for the species. The maximum value of suitability without omission errors was used as a threshold value (as suggested by Rodda *et al.* 2011 for alien species). For model evaluation, the remaining 30% of the data was used to calculate the True Skill Statistics (TSS, Liu *et al.* 2011) and the area under curve ROC (AUC, Fielding and Bell 1997). This analysis was repeated five times, thus providing a fivefold internal cross-validation of models. True Skill Statistics values vary from -1 to +1, so values equal or lower than zero imply statistical fit that is no better than those generated at random. AUC values vary from 0 to 1, values higher that 0.5 being better than random. Final ensembles only included those projections with TSS higher than 0.4 and AUC higher than 0.7. Ensembles were calculated as an average of all these projections, weighted by their AUC values. Final maps show ensembles along water bodies and urban centers.

***Complementary analyses***

To obtain additional measures about the invasive potential of species (in particular, to determine if turtles shift their realized climatic niche during the invasion process), the overlap between native and non-native climatic niches of each species was compared and estimated based on a niche overlap and similarity analysis based on Broennimann *et al.* (2012) available in ecospat package (Broennimann *et al.*, 2012) of R (R Core Team, 2014). This analysis determines statistically if a species is invading areas other than those climatically similar to their native range, which would indicating an even greater potential to spread in the non-native area than that predicted with BEMs.

These analyses were only run for *T. scripta* and *G. pseudogeographica*, given that the implemented database does not contain alien populations of *P. nelsoni*. For each species, non-native niche was characterized on the basis of alien records and native on the basis of natives one. For these analyses, exactly the same background zone (i.e. training area) was used. However, in order to obtain a more complete characterization of the environmental niches (as suggested by Broennimann *et al.* 2012), 19 bioclimatic variables available in Worldclim were used. Finally, information of the main characteristics of habitat, diet and reproduction behavior of each of the species was gathered from the literature (See Table S1 in Supplementary Material).

## Results

Projections of BEMs for the three species had a good performance (see values of AUC and TSS in Fig. 1). Resulted BEMs indicated that Argentina offers large surfaces of suitable climatic space for *G. pseudogeographica* and *T. scripta*. Additionally, in both cases, these climatically suitable areas contain a large area of rivers and water bodies. Considering the "suitability without omission" threshold, 1,341,570 km$^2$ would be suitable for *G. pseudogeographica* and 1,815,207 km$^2$ for *T. scripta*. The suitable surfaces were considerably smaller for *P. nelsoni* (79,530 km$^2$). The northeastern portion of the country would be most climatically suitable for all species (Fig. 1).

The analysis of niche overlap between native and invasive populations showed that the proportion of variance explained by first two PCA axes was high for both species: 80.3% for *G. pseudogeographica* and 81.2 % for *T. scripta*. Niche overlap between native and alien niches was low for both species 0.08 *T. scripta* , and 0.25 for *G. pseudogeographica*. Niche equivalency was rejected for both species, i.e. niche equivalency tests confirmed that niches from the two species in the invaded regions are not identical to the native ones. The niche similarity tests indicate that the niche was more conserved than expected at random for *G. pseudogeographica* only (D = 0.25, similarity test invaded->native: p = 0.009). In contrast, *T. scripta* does not show niche conservatism (D = 0.08, similarity test invaded ->native: p = 0.7). All species are generalists, i.e they are found in a variety of habitats, have a non-specific diet and lay between 20 and 30 eggs in coastal sand banks (see Table S1 in Supplementary Material).

## Discussion

It is not by chance that, in the last three years, the first feral specimens of *T. scripta* have been reported in the country (Alcalde *et al.*, 2012; Prado *et al.*, 2012; Quiroga *et al.*, 2015). Argentinian decision-makers are walking a tightrope: if regulatory policies of the trade and holding of these freshwater turtles are not enforced urgently, new biological invasions could be establishing in the country soon. This is because two of the most imported turtles in the country (*G. pseudographica* and *T. scripta*) have large areas that are climatically suitable, harboring many potentially livable water bodies, in which main ecological resources for the species would be available (both are quite generalist in relation to their diet, reproduction and breeding sites). These areas overlap with important urban centers of the country, which could act as "sources of alien specimens". However, there is still time to change this situation and the fast development of preventive measures could avoid ecological problems, impacts and associated costs (e.g. eradication and habitat restoration) in the near future (Pimentel *et al.*, 2005; Charles and Dukes, 2007; Simberloff *et al.*, 2013). The picture is different for *P. nelsoni*, because climatically suitable areas are smaller for this species, and are far from the most populated areas of the country, where introduction risk is higher. Actually, although this is a highly traded species, as far as we know, there are no reports of established viable populations, suggesting a lower invasive potential.

The situation in the northeast portion of the country is particularly worrisome (including provinces of Corrientes,  Entre Rios, Santa Fe, Misiones and, at lesser extent, Chaco and Formosa), given that the area is highly suitable for all three species (for *P. nelsoni* only in surroundings areas of Parana River and restricted areas of Entre Rios and Corrientes provinces).

In addition, this extent corresponds to the Argentinian Litoral (including humid Chaco and Argentinian Mesopotamia, Olson *et al.* 2001), a region characterized by large rivers and many water bodies (Bonetto *et al.*, 1986), potential habitats for the species to establish and spread. This is also one of the most diverse regions in terms of water-dependent vertebrates in the country, including a portion of Atlantic Forest ecoregion, a Biodiversity Hotspot (Olson *et al.*, 2001; Mittermeier *et al.*, 2004). This region is inhabited by seven species of native freshwater turtles: *Trachemys dorbigni, Mesoclemmys vanderhaegei, Hydromedusa tectifera, Phrynops hilarii, Phrynops williamsi, Phrynops geoffroanus* and *Acanthochelys spixii,* which could be negatively impacted by these alien species (Pearson *et al.*, 2015).

Furthermore, a large portion of Buenos Aires province, with many potentially livable water bodies, is highly suitable for two species (*T. scripta* and *G. pseudogeographica*). This region corresponds with the most populated area of the country, and so introduction rates are likely to be particularly high. Other regions in the northwest portion of Argentina, housing important urban centers such as Tucuman, Salta and Jujuy, are also climatically suitable for these species. The trade and pet ownership of *T. scripta* and *G. pseudogeographica* should therefore also be prohibited in these provinces. Not by chance, 2 out of the 3 reported alien specimens of *T. scripta* in Argentina were found there (Alcalde *et al.*, 2012; Prado *et al.*, 2012). Fortunately, these specimens were quickly removed by the authors of the mentioned papers (i.e. Prado et al. and Alcalde et al.). However, given the antecedents, these areas (and those highly suitable in the northeast of the country) should be systematically monitored and the individuals of the species removed from wildlife in order to avoid potential establishment and its associated problems.

As stated in the introduction, alien individuals of *Trachemys* are very prone to hybridizing when in contact with related species, generating genetic pollution, offspring with a low fitness and potentially the loss of native genotypes (Parham *et al.*, 2013; Figuereido, 2014). This is a major threat for the native populations of *T. dorbigni,* given the proximity with the reported feral *T. scripta* specimens (in northeastern portion of Buenos Aires province)(Alcalde *et al.*, 2012; Prado *et al.*, 2012). It is very important to undertake strict monitoring in those areas, aimed at finding and removing new specimens. Moreover*,* genetic analyses are also needed to test the potential presence of hybrid specimens.

In relation to the climatic tolerance of the species, comparisons between invasive and native climatic niches revealed niche conservatism for *G. pseudogeographica* (Fig. 2; see also Broennimann *et al.* 2012; and Guisan *et al.* 2014 for details). This result indicates that the native climatic niche of the species reflects its climatic tolerance (similar results have been found for others invasive species, e.g. Palaoro *et al.* 2013; Faleiro *et al.* 2015). However, results of this study also highlighted the vast alien climatic niche of *T. scripta* and suggested that this species has the potential to establish alien populations in places that do not represent native climatic tolerance of the species ( Gallagher *et al.* 2010). This result is highly consistent with those recently found by Rodrigues *et al.* (2016) providing additional evidence that this species has the potential to change its climatic niche when a new area is invaded, a clear proof of the great invasive potential of this species (Fig. 2). Such shift of realized niches has major consequences. The areas with suitable climate for *T. scripta* in Argentina have been identified using BEMs mostly developed with native records. Such shift of niches would suggest that the areas identified by BEMs may be an underestimation of suitable areas, and that. the species could be able to establish viable populations in areas which are not predicted in the final map of this study.

Based on the results of BEMs, niche overlap analyses and ecological characteristics of the species, particularly vulnerable places have been determined, and important recommendations for the regulation of trade of the most imported freshwater turtles in Argentina have been suggested. Although all three species have the potential to establish viable populations in the country, results of this study highlight that the importation trade and breeding of *T. scripta* should be strictly banned in the entire national territory because, given its great invasive potential, the species could establish viable populations across very large areas of the country. Additionally, the trade and breeding *G. pseudogeographica* must be prohibited in all of the north and central provinces of the country. Finally, the income and trade of any of the three species must be forbidden in Corrientes, Misiones and Entre Rios provinces where the most vulnerable freshwater ecosystems are located. Recommendations of this study should be strongly considered given that an invasion of any of these species could represent important ecological problems and future economic losses, associated with the eradication, habitat restoration and potential degradation of ecosystem services (see introduction, and Simberloff *et al.*, 2013).

Fortunately, Argentinian decision-makers seem to be increasingly aware about potential invaders in general. In fact, around 5% of the national territory (2 of the 23 political provinces) has sanctioned policies that establish strong limitations to the trade and possession of non-native species (e.g. environmental provincial law 7343 of Cordoba Province, Argentina). Hopefully most decision-makers in the country will take these policies as an example, at least for specific species in "key sites" as those reported here. Of course, interaction between science and decision-makers should take a leading role in the prevention of these kinds of ecological problems.

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**Figure legends**

**Figure 1:** Maps showing final projections of the ensembles for each species in Argentina. Red gradient represents the climatic suitability of each pixel. Circles represent urban centers. Rivers and water bodies are represented by light blue polygons.

**Figure 2:** Each individual panel represents the niche of the species along the two first axes of the PCA in the North American native niche (up) and non-native niche (down). Grey shading shows the density of the occurrences of the species by cell. The solid and dashed contour lines illustrate 100% and 50%, respectively, of the available (background) environment.

## Supplementary material legends

**Figure S1:** Records of each species implemented for the analyses. Native records (red triangles) within the IUCN extent (yellow) and alien populations discriminating each source of information are shown.

**Table S1:** Summary of the main characteristics of habitat, diet and reproduction of the studied species, and associated references.