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PLANTS IN AQUATIC SYSTEMS

Prioritisation of aquatic invasive alien plants in South America with the US Aquatic Weed Risk Assessment

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Abstract Forty South American aquatic plant species were selected and categorised in four a priori status classes (alien naturalised, alien invasive, native and absent) according to expert opinion, for 16 South American regions (Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Falklands Islands, French Guiana, Galapagos, Guyana, Paraguay, Peru, South Georgia and South Sandwich Islands, Suriname, Uruguay and Venezuela). The 40 aquatic plant species were assessed using the US Aquatic Weed Risk Assessment (USAqWRA) scheme for each of the 16 South American regions, for a total number of 644 assessments and for South America (153 assessments). The method was benchmarked against expert opinion (invasive, non-invasive). We ranked 17 of them as naturalised, and 15 as invasive species in at least one South American region. The USAqWRA distinguished between non-invaders and invaders with an overall accuracy of 84.9% in South America and 54.1% in the 16 regions, with areas under the curves equal to 0.893 and 0.853, at a threshold score of 51.5 and 43.5, respectively. The study highlights that the USAqWRA could represent a suitable screening protocol to prioritise aquatic species that have the potential to cause negative impacts, prevent attempts of introduction and to manage risky aquatic plants in South America.

Keywords Negative impacts · Non-native aquatic species · Aquatic plants · Risk assessment · Prioritisation

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Introduction

Freshwater ecosystems, in particular lakes and reservoirs, have been identified to be both highly vulnerable to invasive species (Strayer, 2010; Simberloff, 2013; Boltovskoy & Correa, 2015) and the most endangered ecosystems in the world (e.g., Collen et al., 2014). Biological invasions in freshwaters can be dramatic because freshwater ecosystems have the greatest concentration of species per surface area in the planet (Thomaz et al., 2015) and they act as stepping stones for establishing invaders in new watersheds (Havel et al., 2015). At the same time, aquatic and semi-aquatic plants have a higher probability of becoming invasive than do species from terrestrial plant families (Daehler, 1998) and thus form a significant proportion of potential invasive species (Andreu & Vilà, 2010; Azan et al., 2015).

The excessive growth of invasive alien macrophytes can produce negative impacts on the invaded freshwater ecosystem and substantially change the hydrology, sedimentation, water clarity and nutrient state of river and lakes (Gallardo et al., 2015; Havel et al., 2015). Aquatic invasion may, for example, reduce the habitat available for other species positioned higher in the trophic web such as invertebrates and fish (van Kleunen et al., 1999; Matsuzaki et al., 2009; Carniatto et al., 2014).

In order to effectively prioritise management options, stakeholders affected by biological invasions need to be able to identify those species, among different taxa, that are likely to cause the most damage (Hulme et al., 2012; Kumschick et al., 2015). Non-native species are not uniformly invasive nor harmful (Santos et al., 2011), and may have a little or undetectable impact in the new region or produce negative impacts only after a certain period of time (Pyšek et al., 2012; Strayer, 2012) as "sleeping weeds" (Groves, 2006). These alien plants can behave as minor invaders for decades before they become serious invaders. Nevertheless, impacts may vary along time and among species and regions. In the framework of this research is essential not to understate the risk of potential impacts from species that may have delayed invasions.

The scarcity of studies on plant invasions both in terrestrial and aquatic ecosystems and the analogous paucity of supporting policy and investment in Latin America pose an opportunity to develop an invasive plant research agenda specifically focused on South America, to provide knowledge to help identify priorities for both decision makers and managers (Gardener et al., 2012). Impacts of invasive alien plants are not always perceived as such and they may differ throughout the South American region.

In South America, Chile, offers a unique opportunity to study biological invasions because it has a unique native flora with high levels of endemism, extraordinary richness and diverse climatic gradients (Pauchard et al., 2004). In addition, Chile and Brazil have been suggested as the very suitable regions to test invasion ecology generalities and hypotheses that have been tested in other parts of the world (Ormazabal, 1993; Arroyo et al., 2000; Myers et al., 2000). The Guiana Shield (Guyana, Suriname and French Guiana) constitutes a geological, hydrographical and biogeographic region in the Amazonian Basin that is considered a biodiversity hotspot (Delnatte & Meyer, 2012) and the Galapagos island is another major hotspot particularly vulnerable to invasions by alien species, which now present the largest threat to terrestrial biodiversity (Trueman et al., 2010). South America offers a large variety of water bodies and habitats for macrophytes with many large river systems and streams cross many countries. Argentina, Brazil and Paraguay represent a clear example of interconnecting transboundary water bodies like the Paraná River Floodplain, where freshwater wetlands cover 3650 km^2. These networks provide opportunities of natural spread of aquatic plants in areas previously free of alien vegetation.

The accelerating worldwide movement of people and human activities are driving the increasing rate at which biological invasions are occurring (e.g., Essl et al., 2011; Seebens et al., 2013; Essl et al., 2015) and South America is not an exception to this trend (e.g., Almeida et al., 2015). Currently, introductions of non-native plants caused by human-presence represent 45% of total plant species on Galapagos (Mauchamp, 1997; Guézou et al., 2010; Heleno et al., 2013). Trade and cross-border connections are, for example, constantly increasing between Brazil, French Guiana, Suriname, and Guyana. The cross-border cooperation program for the 2014–2020 period between the outermost region of French Guiana, Suriname and the states of Amapá and Amazonas in Brazil is expected to double the number of passengers (currently nearly 12,000) and triple the number of vehicles (currently 7800) crossing the Maroni by ferry each year (European Commission, 2015). Similarly, anthropogenic disturbances may contribute favouring naturalisation and invasion of intentionally or accidentally introduced alien plant species (Delnatte & Meyer, 2012). Bini & Thomaz (2005) reported a large number of aquatic weeds that were introduced in Paraná River, Brazil, affecting electric power generation. Fuentes et al. (2010) remarked how trade between Chile and Argentina may facilitate the transport of propagules, thus increasing the risk of new alien plant introductions.

Prioritisation, Risk Assessment and Risk Analysis are fundamental tools for managing non-native species and identifying those species that are likely to become invasive and cause significant negative impacts (e.g., Brunel et al., 2010; Kumschick et al., 2012; Verbrugge et al., 2012). Among risk assessment schemes, the Australian Weed Risk Assessment (A-WRA, Pheloung et al., 1999) was shown to be effective in classifying plant invaders across several islands and continents (Gordon et al., 2008). The A-WRA is routinely used for regulatory purposes in Australia, New Zealand and Chile. The A-WRA has been adapted to other parts of the world including Hawaii (Daehler & Carino, 2000), Hawaii and Pacific Islands (Daehler et al., 2004), Czech Republic (Křivánek & Pyšek, 2006) and Bonin Islands (Kato et al., 2006). A-WRA has also been used to help manage quarantine issues between countries that share a land border, such as Chile and Argentina (Fuentes et al., 2010) and guidance questions could easily be modified to suit the needs in Latin America (Gardener et al., 2012).

However, many of the A-WRA questions are specific to terrestrial plant species, therefore, this scheme is considered less accurate in discriminating between aquatic invaders and non-invaders considered at least at the US scale (Gordon & Gantz, 2011). More recently, the Aquatic Weed Risk Assessment Model (AWRAM) was developed for New Zealand (NZAq-WRA) (Champion & Clayton, 2000, 2010) and subsequently it has been applied in Australia and Micronesia (Champion et al., 2008; Champion & Clayton, 2010). Gordon et al. (2012) developed a modified AWRAM scheme for USA called US Aquatic Weed Risk Assessment (USAqWRA).

As far as we know, our study is the first attempt to apply a specific risk assessment scheme (USAqWRA) for aquatic plants in South America. Therefore, the present research aims to benchmark the USAqWRA on a group of 40 aquatic plant species, across 16 South American regions, comparing its scoring and classification with the existing a priori classification of the invasive status based on South American expert opinion.

Methodology

Study area and species selection

The present research focuses on 40 aquatic plant species. Among them, four a priori status categories were defined according to expert opinion, classifying each of the forty species in one of the four following status categories for each of the 16 regions defined in the present study, or for part of the regions. The four status categories were as follows: alien naturalised (NNV),[1] alien invasive (INV),[2] native (IND) and absent (ABS) (e.g., Richardson et al., 2000; Pyšek et al., 2004, 2009). To perform data analysis, these four a priori status categories were grouped in an additional binary category: invasive and non-invasive, the latter including both alien naturalised (but not invasive) and native species. In addition, for each of the 40 species, we assigned a priori status for the entire region of South America, as a binary category: invasive and non-invasive. This South American status was based as well on expert opinion, taking into account the worst scenario, i.e. a species was categorised as invasive in South America whenever it was considered invasive in at least one of the 16 regions; otherwise it was considered as non-invasive in South America. Therefore, species only naturalised but non-invasive were included in this second category.

The invasive alien species are those reported as naturalised with negative ecological impacts on biodiversity, economy, and ecosystem services according to local experts' opinion. Local experts where contacted by e-mail. They were asked to provide list of aquatic species for the region of their expertise, specifying the biogeographic status (alien/native) and the invasive status (invasive/non-invasive), as well as all the available scientific and grey literature. Our data collection included both helophytes (plants in which surviving buds are buried in water-saturated soil, or below water-level, but that have flowers and leaves that are fully emergent during the growing season; it includes emergent aquatic herbs) and hydrophytes (fully aquatic herbs in which surviving buds are

[1] Naturalised: alien plants that sustain self-replacing populations for at least 10 years without direct intervention by people (or in spite of human intervention) by recruitment from seed or ramets capable of independent growth, and do not necessarily invade natural, seminatural or human-made ecosystems (Richardson et al., 2000; Pyšek et al., 2004; Blackburn et al., 2011).

[2] Invasive: subset of naturalised plants that produce reproductive offspring, often in very large numbers, at considerable distances from the parent plants (approximate scales: >100 m in <50 years for taxa spreading by seeds and other propagules; >6 m in 3 years for taxa spreading by roots, rhizomes, stolons, or creeping stems), and thus have the potential to spread over a large area. (Richardson et al., 2000; Pyšek et al., 2004; Blackburn et al., 2011).

submerged, or buried in soil beneath water; their stems and vegetative shoots growing entirely underwater with leaves submerged or floating, but only the flower-bearing parts emergent, see Raunkiaer 1934, as modified by Govaerts et al. 2000), and can be classified as free-floating, floating (rooted), emergent and submerged freshwater macrophytes (Table 1). We also cross-checked literature and databases on the status of the species reported in each region by experts (Table 1 of Appendix I—Supplementary Material).

The 16 South American regions (there after called "regions") are defined as follows: (1) Argentina, (2) Bolivia, (3) Brazil, (4) Chile, (5) Colombia, (6) Ecuador, (7) Falklands Islands, (8) French Guiana, (9) Galapagos, (10) Guyana, (11) Paraguay, (12) Peru, (13) South Georgia and South Sandwich Islands, (14) Suriname, (15) Uruguay and (16) Venezuela.

Risk assessment methodology

The USAqWRA scheme is a modified version by Gordon et al. (2012) of the original NZAqWRA scheme (New Zealand Aquatic Weed Risk Assessment). The USAqWRA addresses questions on ecology, competitive ability, dispersal modes, reproductive capacity and mode, potential for different types of impacts (e.g., hindrance to navigation, water quality), resistance to management, and history of invasion elsewhere. After answering the 38 questions, which are divided into 12 groups, the protocol assigns a final score as a sum of the values for each question. The final score can range between 3 and 91, with higher scores indicating species with a higher risk.

We calculated the USAqWRA total score for each of the 40 aquatic plants and for each South American region or part of region for a total number of 644 assessments (Table 2 of Appendix I—Supplementary Material). For example, *Catabrosa aquatica* (L.) P.Beauv., was assessed twice both for Argentina and Chile, as it is considered both as native in one part and non-native and non-invasive in another part of the region (Soreng & Fish, 2011). Similarly, *Egeria densa* Planch., is recorded both as native and alien invasive in different regions of Brazil (Rodrigues & Thomaz, 2010; Aona et al., 2015).

Among the 644 assessments, we selected a subset of 153 assessments, according to the following criteria: for each species we took into account the worst scenario for the whole 16 regions and selected all the assessments in accordance; i.e. if a species was invasive in one or more regions we selected one or more assessments accordingly, if a species was not invasive in any region we selected the assessments for the regions where it was considered at least naturalised.

The USAqWRA questions were answered using information from a variety of sources including online databases and factsheets (i.e. http://plants.jstor.org; http://www.ville-ge.ch/cjb; http://www.eppo.int; http://www.nobanis.org; http://www.issg.org; http://plants.usda.gov; http://www.floraargentina.edu.ar; http://floradobrasil.jbrj.gov.br; http://www.tropicos.org). We collected all the available literature from Scopus, Web of Science, Google Scholar and Research Gate using specific key words and search term combinations: (invasive aquatic species OR aquatic invasion) AND (alien plant OR plant invasion OR exotic plant) AND (South American invasion OR South American macrophytes). Data about invasiveness from outside South America were used to answer questions about invasiveness. Nevertheless, when considering the questions 11, 27–29, 32 and 33–37 (noted in Results Table 3) in the USAqWRA scheme, we scored differently on a case by case basis, taking into account the native/alien status of the assessed species in that specific region.

In order to test the difference between the a priori binary status for the 16 regions and for South America (invasive vs. non-invasive), we used One-way Analysis of Variance (ANOVA).

Evaluation of the performance of USAqWRA scheme

The whole set of 644 assessments was considered for evaluating the performance of the USAqWRA scheme for each of the 16 regions, while the subset of 153 assessments was used to evaluated the scheme at South American level.

The performance of the USAqWRA was benchmarked using Receiver Operating Characteristic (ROC) curve analysis and compared to the expert opinion (invasive vs. non-invasive), respectively, for the 40 aquatic species for each region (644 assessments) and for South America (153 assessments). This method is widely used for assessing the performance of a screening test. A ROC curve represents test specificity (accuracy for correctly categorising non-

Table 1 List of the investigated 40 aquatic plant species varying for native origin

Species	Native origin	Life span	Life form	Pathway of introduction		Expert opinion range	USAqWRA score range (min–max)
Alisma lanceolatum	E/NAf/A	P	Hy/E	Y		ABS, NNV	32
Alisma plantago-aquatica	Aus/E/A	P	Hy/S	Y		ABS, NNV	32
Alternanthera philoxeroides	SA	P	He	Y	S/Ballast water	ABS, IND, NNV	51–63
Arundo donax	A/E	P	He	Y		ABS, INV	66–69
Azolla filiculoides	SA/CA/NA	AP	Hy/FF	Y	S/Ballast water	ABS, IND, INV	39–54
Brachiaria subquadripara	Tropics	AP	Hy/E	N	C/Forage	ABS, INV	49–52
Catabrosa aquatica	Circumboreal/SA	P	Hy	NA		ABS, IND, NNV	14–19
Ceratophyllum demersum	Cosmopolitan	P	Hy/S	Y		ABS, IND, INV	28–39
Crassula peduncularis	Aus/NZ/SA	A	He	Y		ABS, IND	20–23
Cyperus difformis	E/Af/A	A	He	N	S/Machinery-equipment	ABS, INV	41–44
Echinodorus uruguayensis	SA	P	Hy/S	NA		ABS, IND	25–28
Egeria densa	SA/E	P	Hy/S	Y		ABS, IND, INV	54–66
Egeria najas	SA	P	Hy/S	NA		ABS, IND, INV	36–51
Eichhornia azurea	SA	P	Hy/F	NA		ABS, IND	49–61
Eichhornia crassipes	SA	P	Hy/FF	Y		ABS, IND, INV	66–76
Elodea canadensis	NA	P	Hy/S	Y		ABS, NNV	39
Hippuris vulgaris	E/NA/SA	P	Hy/S	Y		ABS, IND, INV	38–46
Hydrilla verticillata	A/Af	P	Hy/S	Y		ABS, INV	59–61
Hydrocleys nymphoides	SA	P	Hy/F	Y		ABS, IND, NNV	30–37
Hydrocotyle leucocephala	SA	P	Hy/F	NA		ABS, IND	34–47
Hydrocotyle ranunculoides	SA/CA/NA	P	Hy/E	Y		ABS, IND, INV	47–62
Lemna gibba	NA/E/A/SA	AP	Hy/FF	NA		ABS, IND, NNV	36–46
Lemna minor	Cosmopolitan	P	Hy/FF	Y		ABS, IND, NNV	40–49
Limnobium laevigatum	SA/CA	P	Hy/FF	NA		ABS, IND, INV	45–60
Ludwigia grandiflora	NA/SA	P	He	Y		ABS, IND	51–68
Ludwigia peploides	NA/CA/SA	P	He	Y		ABS, IND, INV	49–64
Myriophyllum aquaticum	SA	P	Hy/S	Y		ABS, IND, NNV	53–63
Myriophyllum quitense	SA	P	Hy/S	NA		ABS, IND	28–45
Nymphaea alba	E/Es	P	Hy/F	Y		ABS, INV	30–33
Nymphaea lotus	A/Af	P	Hy/E	Y		ABS, NNV	39
Nymphoides indica	Subcosmopolitan	P	Hy/F	Y		ABS, IND, NNV	23–28
Pistia stratiotes	SA	P	Hy/FF	NA		ABS, IND, NNV	54–67
Potamogeton pusillus	Cosmopolitan	AB	Hy/S	NA		ABS, IND, NNV	28–30
Ranunculus aquatilis	E/A/Am/Aus/Af	AP	Hy/S	Y		ABS, IND, NNV	22–27
Sagittaria guayanensis	SA	P	Hy/E	NA		ABS, IND	24–35
Salvinia auriculata	SA/CA	A	Hy/FF	NA		ABS, IND, NNV	44–55
Salvinia molesta	SA	P	Hy/FF	Y		ABS, IND, NNV	49–64
Spirodela punctata	Aus/East A	P	Hy/FF	Y	S/Ballast water	NNV	24
Typha angustifolia	Circumboreal	P	Hy/E	N	S/Machinery-equipment	ABS, INV	39
Wolffia Braziliensis	SA	P	Hy/FF	NA		ABS, IND	18–27

(*Af* Africa, *Am* America, *Aus* Australia, *A* Asia, *E* Europe, *Es* Eurasia, *NA* North America, *NAf* North Africa, *SA* South America), status according to expert opinion in South America (*INV* alien invasive, *NNV* alien non-invasive, *IND* native, *ABS* absent) and different life span and life forms (*A* annual, *AB* annual/biannual, *AP* annual/perennial, *P* perennial, *He* helophyte, *Hy* hydrophyte, *E* emergent, *F* floating rooted, *FF* free-floating, *S* submerged). Pathway of introduction is shown as a category and subcategory: *Y* Escape (the species was voluntary introduced as an ornamental), *N* pathway not related to the use as an ornamental species, *NA* information not available. Other pathway categories: *C* contaminant, *S* stowaway. The USAqWRA scores are shown as a range with minimum and maximum values invasive plants as having low risk of invasiveness, i.e. true negatives) against the complement of sensitivity (accuracy for correctly categorising invasive plants as having high risk of invasiveness, i.e. true positives) over the range of potential cut-off levels (Conser et al., 2015).

The areas under the ROC curves (AUROC) were calculated using the software R (R Core Team, 2015) and the R package "pROC" (Carstensen et al., 2015). An AUC value closer to 1.0 would indicate that the scheme perfectly discriminates between invaders and non-invaders. On the contrary, values near 0.5 indicate an inability to discriminate (Conser et al., 2015). Threshold USAqWRA score was calculated by the point of the ROC curve closest to the point on the axes that maximises the true positives and minimises the false positives.

In addition, we used the R package "lme4" (Bates et al., 2015) to perform a generalised linear mixed models (GLMM) analysis of the relationship between the USAqWRA scores (invasiveness risk) and the expert assessment status (alien invasive, alien non-invasive, native, absent), for each aquatic species and in each SA region (or part of region). The GLMM analysis was considered the most suitable method because the response variable of interest (USAqWRA scores) was not normally distributed (Shapiro–Wilk normality test, $W = 0.97335$, P value $= 2.007e{-}09$) and the Levene's test did not support the presence of equal variances. Furthermore, our USAqWRA scores were repeated measures (estimates) on the same set of species (40) and regions (16). Mixed models allow including both fixed and random variables, which is required in studies where individuals are repeatedly measured (Faraway, 2006; Bolker et al., 2009; Hamel et al., 2016). As fixed effects, we entered into the GLMM model the plant species (40) and the expert assessment of the status (4 categories), without interaction terms. As random effects, we had intercepts for species:status:regions. This model was selected among the other possible models (including the null model with no fixed effects and only species as random effect and a full model with three fixed effects), taking into account its lower AIC score (i.e. 118.55 vs. 453.98 for the null model).

Evaluation of the performance of the single questions of the USAqWRA scheme

To determine which questions of the USAqWRA scheme contributed to the predictability of invasiveness versus non-invasiveness, we applied a logistic regression model, considering as a dependent binary variable the successful outcomes of the USAqWRA scores, i.e. the sum of true positive and true negatives versus the sum of false positives and false negatives (unsuccessful outcomes, over 644 assessments). Due to the large number of questions, to avoid convergence problems between parameters (for the maximum likelihood estimation, see Heinze & Ploner, 2003, 2004), Firth's bias reduced logistic regression was used as implemented in the R package "logistf" (Heinze et al., 2013). For each question, we calculated the percentage of times it was answered both for invasive and non-invasive aquatic species.

Results

Species status categories according to expert opinion

As a result, among the 40 investigated species, 17 are naturalised alien and 15 are invasive alien in at least one of the 16 South American regions considered in this study. Eight species are native to one or more regions, but are absent in the other regions and therefore they potentially could be introduced in the future. Using terminology in line with the EU project DAISIE (Pyšek et al., 2009), we can highlight that only 6 of the 15 invasive alien species and 5 of the 17 naturalised ones are *alien to* South America (i.e. with a native range outside SA), while the other 9 invasive and 12 naturalised species are *alien in* South America, i.e. with a SA origin but occurring as alien in other parts of the SA continent. While this sample size was relatively small, it included different categories of aquatic plant species which represent diversity in both taxonomic relationship, phenology, life form and level of risk according to expert opinion (Table 1 and Supplementary materials).

According to expert opinion, the regions with the larger numbers of alien invasive aquatic plant, among the forty species investigated in the present study, are as follows: Chile (9 species), Brazil (6) and Colombia (3) (Fig. 1). The regions with the larger numbers of alien non-invasive aquatic plants are as follows: Chile (16), Argentina (11) and Colombia (10). In two of the study regions, i.e. in Argentina and Bolivia, 21 of the 40 investigated species are considered native

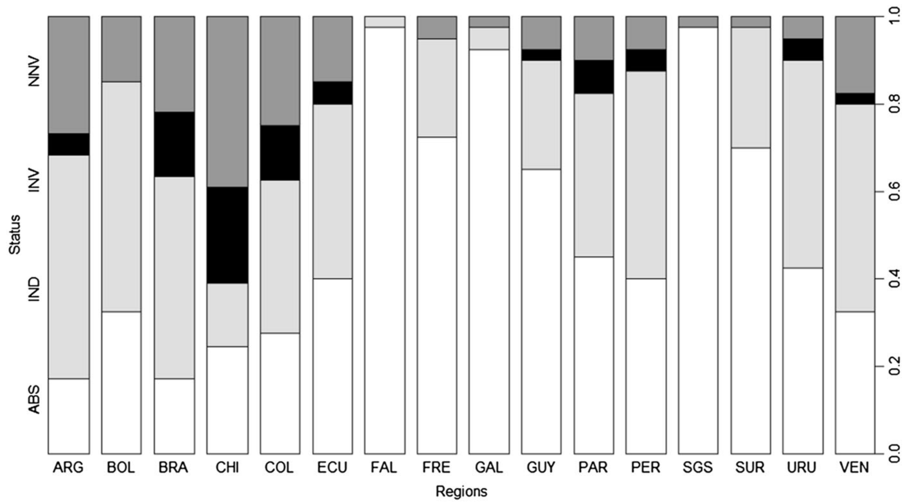

Fig. 1 Regional distribution of the four a priori status defined by expert opinion of the 40 South American aquatic plant species investigated (*ABS* absent, *IND* native, *INV* alien invasive, *NNV* alien naturalised and non-invasive)

according to expert opinion (Fig. 1). None of the 40 aquatic plant species investigated are present in the Falkland Islands, with the exception of *Myriophyllum quitense* Kunth which is considered native to the archipelago.

Noteworthy, *Eichhornia crassipes* (Mart.) Solms is considered invasive in 10 South American regions and native in 4 regions, while *Arundo donax* L., is considered invasive in 4 regions, *Cyperus difformis* L., and *E. densa* Planch., in 3 regions. All the other species are considered invasive in a lower number of regions, or they are considered alien but non-invasive or native (Table 1 and Supplementary materials).

USAqWRA scores and invasion categories

We assessed the 40 South American aquatic plant species in each of the 16 regions (or part of a region) for a total of 644 assessments. The information collected for each species allowed us to answer a mean of 37 questions (range 34–38 questions, ±1.3 SD) out of 38 questions of the USAqWRA scheme, for each of the 644 assessments.

The scores obtained by the 15 species classified as invasive by expert opinion in at least one region (INV) ranged from 30 to 76 (33 assessments); the scores for the 17 species classified as non-invasive alien (NNV) ranged from 19 to 67 (82 assessments); the scores for the 8 species classified as native (IND) ranged from 14 to 66 (203 assessments). The other 326 assessments were done for those species not present in a region (ABS), therefore for all regions at potential risk of invasion, and scores ranged from 14 to 73.

The difference among invasion categories (invasive and non-invasive) were highly significant both in 16 regions (P value = 4.197e−16) and in South America (P value = 2.2e−16) (Fig. 2).

The four species with the highest scores were (76) *E. crassipes*; (69) *A. donax*; (68) *Ludwigia grandiflora* (Michx.) Greuter & Burdet, and (67) *Pistia stratiotes* L. (Table 1). The lowest USAqWRA scores were 14–19 and 18–27, respectively, for *C. aquatica* and *Wolffia iensis* Wedd. The lowest score for *C. aquatica* (14) refers to Argentina and Chile being considered native to part of these regions, and to the 14 regions where it is absent. On the contrary, the highest score for *C. aquatica* (19) refers to the parts of Argentina and Chile where this species is considered a naturalised alien. In the case of *W. brasiliensis*, the lowest score (18) was obtained for the 11 regions where the species is considered native, while the highest score (27) was obtained for the 5 regions where the species is presently absent (Table 2 Appendix I—Supplementary Material).

The results of the GLMM analysis for fixed effects are displayed in Table 2. These results clearly state that the USAqWRA scores are significantly correlated (positively or negatively) for 32 of the 40 aquatic species assessed (Table 2).

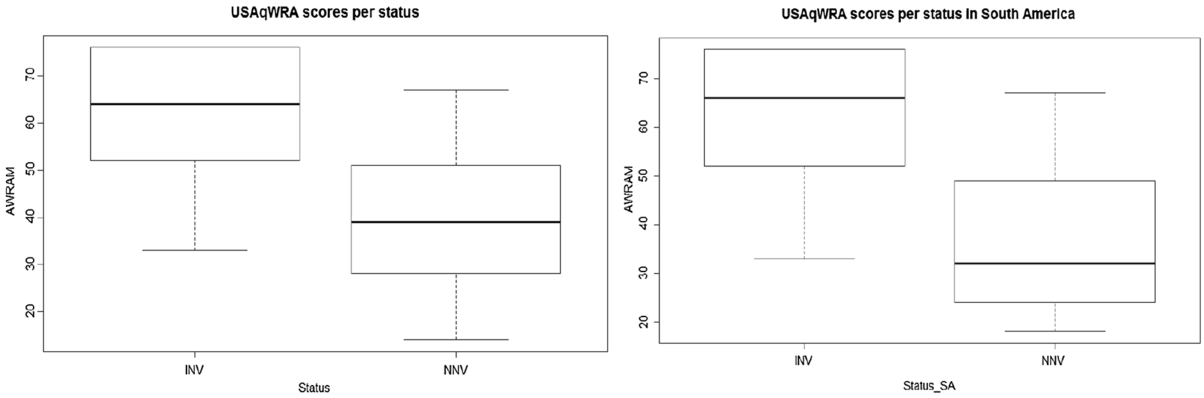

Fig. 2 *Box* and *whisker* plots showing USAqWRA scores of the 40 South American aquatic plant species. On the *left* the results for 16 regions (644 assessments), on the *right* for South America as a single continental unit (153 assessments), for the two categories of invasion: alien invasive (INV) and alien naturalised (non-invasive), including native species (NNV)

Accuracy of the USAqWRA scheme

The USAqWRA distinguished between non-invaders and invaders with an overall accuracy of 54.1% and an AUC = 0.853, at a threshold score of 43.5 (point on the ROC curve that maximised the ability for classification of the test). The percentage of specificity (54.17%) was slightly lower than the sensitivity (54.54%).

In addition, on the subset of 153 assessments, we obtained for South America a higher overall accuracy of 84.9% and a larger AUC = 0.893, at a threshold score of 51.5 (Fig. 3). The percentage of specificity (87.50%) was higher than the sensitivity (75.75%).

Species with USAqWRA scores higher than the threshold are predicted as having a high risk of invasiveness while species with scores lower than the threshold are predicted as a non-invasive or at lower risk, respectively at region level and at South American level.

USAqWRA questions and their predictive power

To identify those questions having a significantly higher predictive power to separate invasive aquatic plants from non-invasive, we used a penalised likelihood based method called Firth logistic regression. A group of 8 questions (with a $P < 0.05$ over the 38 questions of the scheme) was delimited. These questions had a higher significant predictive power to separate invasive aquatic plants from non-invasive aquatic plants, although the percentage of time each question was answered was lower (for non-invasive species) (Table 3). These questions can be classified into three groups: (1) ecology and habitat of the species; (2) seeding ability; and (3) potential of impact and damage to natural areas. In Table 3, the percentages of times each of the 38 questions was answered are shown. The percentages for invasive plants ranged from 93 to 100% and for non-invasive plants ranged from 76 to 100%.

Discussion

As far as we know, this is the first application of the USAqWRA scheme to South America. Furthermore, only very few aquatic plant species have been risk assessed in South American countries using any other risk assessments method, as in the case of those assessed with the Weed Risk Assessment for Chile and Argentina by Fuentes et al. (2010) (Supplementary materials). In the present research, we assessed 40 aquatic species using the USAqWRA concluding that the method can be conveniently applied to South American aquatic plant species, when there is enough available knowledge on the assessed species. At the same time, the USAqWRA score can be used to prioritise species according to their level of risk.

As remarked in the methodology section, the available expert opinion allowed the identification of only four a priori status categories that were subsequently grouped in a binary invasion category (invasive and non-invasive). Therefore, we were in a

Table 2 Results of the GLMM analysis for the fixed effects considered in the model

Fixed effects	Estimate	SE	*Z* value	*Pr*(>|z|)	Sign. code
(Intercept)	3.47E+00	4.42E−02	78.39	<2.00E−16	***
Alisma plantago-aquatica	3.60E−05	6.25E−02	0.00	0.99954	
Alternanthera philoxeroides	6.89E−01	5.64E−02	12.22	<2.00E−16	***
Arundo donax	7.19E−01	5.55E−02	12.94	<2.00E−16	***
Azolla filiculoides	4.46E−01	5.95E−02	7.49	6.68E−14	***
Brachiaria subquadripara	4.25E−01	5.69E−02	7.47	8.13E−14	***
Catabrosa aquatica	−7.65E−01	7.60E−02	−10.06	<2.00E−16	***
Ceratophyllum demersum	3.39E−02	6.30E−02	0.54	0.590222	
Crassula peduncularis	−3.00E−01	6.96E−02	−4.31	1.65E−05	***
Cyperus difformis	2.44E−01	5.95E−02	4.09	4.25E−05	***
Echinodorus uruguayensis	−1.01E−01	6.54E−02	−1.54	0.12356	
Egeria densa	6.56E−01	5.46E−02	12.03	<2.00E−16	***
Egeria najas	3.66E−01	5.83E−02	6.28	3.49E−10	***
Eichhornia azurea	6.54E−01	5.74E−02	11.4	<2.00E−16	***
Eichhornia crassipes	8.57E−01	5.62E−02	15.24	<2.00E−16	***
Elodea canadensis	1.98E−01	5.98E−02	3.31	0.000925	***
Hippuris vulgaris	3.04E−01	5.84E−02	5.2	1.96E−07	***
Hydrilla verticillata	6.09E−01	5.50E−02	11.07	<2.00E−16	***
Hydrocleys nymphoides	1.56E−01	6.26E−02	2.5	0.012461	*
Hydrocotyle leucocephala	3.39E−01	6.00E−02	5.65	1.60E−08	***
Hydrocotyle ranunculoides	6.15E−01	5.60E−02	10.98	<2.00E−16	***
Lemna gibba	3.59E−01	5.89E−02	6.09	1.12E−09	***
Lemna minor	4.32E−01	5.75E−02	7.52	5.46E−14	***
Limnobium laevigatum	5.79E−01	5.80E−02	9.97	<2.00E−16	***
Ludwigia grandiflora	7.26E−01	5.56E−02	13.05	<2.00E−16	***
Ludwigia peploides	6.48E−01	5.68E−02	11.42	<2.00E−16	***
Myriophyllum aquaticum	6.84E−01	5.53E−02	12.36	<2.00E−16	***
Myriophyllum quitense	2.10E−01	6.21E−02	3.38	0.000725	***
Nymphaea alba	−6.59E−02	6.36E−02	−1.04	0.300309	
Nymphaea lotus	1.98E−01	6.00E−02	3.3	0.000961	***
Nymphoides indica	−1.11E−01	6.79E−02	−1.63	0.102099	
Pistia stratiotes	7.50E−01	5.66E−02	13.27	<2.00E−16	***
Potamogeton pusillus	7.53E−04	6.44E−02	0.01	0.990667	
Ranunculus aquatilis	−1.67E−01	6.58E−02	−2.54	0.011059	*
Sagittaria guayanensis	−1.06E−02	6.66E−02	−0.16	0.873466	
Salvinia auriculata	5.47E−01	5.79E−02	9.44	<2.00E−16	***
Salvinia molesta	6.88E−01	5.46E−02	12.6	<2.00E−16	***
Spirodela punctata	−2.87E−01	6.83E−02	−4.21	2.57E−05	***
Typha angustifolia	1.97E−01	5.99E−02	3.28	0.001035	**
Wolffia iensis	−2.77E−01	7.12E−02	−3.89	0.000101	***
Native	−2.32E−01	1.70E−02	−13.63	<2.00E−16	***
Alien invasive	2.27E−02	2.83E−02	0.8	0.423226	
Alien non-invasive	−5.77E−04	2.05E−02	−0.03	0.977564	

The significant *P* values are reported and graphically coded in the last column (0 '***' 0.001 '**' 0.01 '*' 0.05 '.'). The Species:Status:Country Intercept values (random factor) equal to 2.566e−18 and 1.602e−09

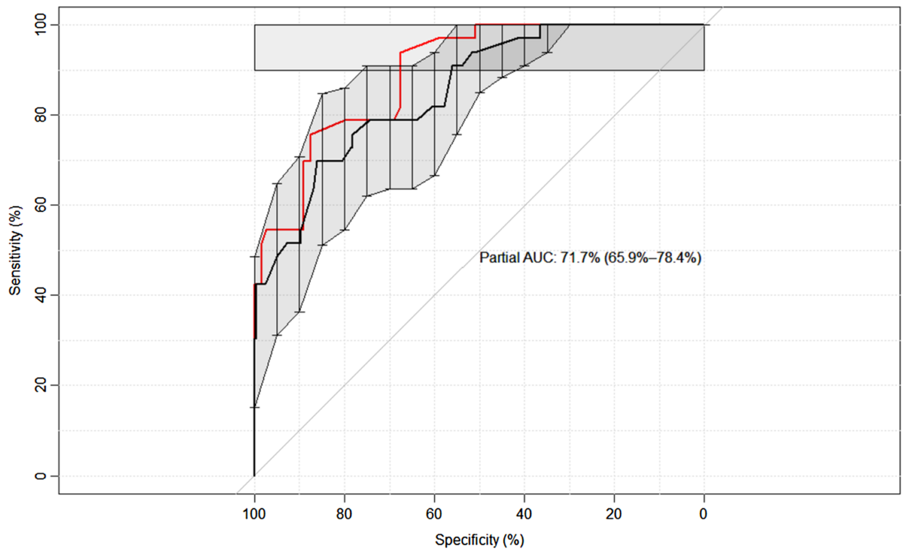

Fig. 3 Receiver operating characteristic (ROC) curves graph of the performance of the USAqWRA scheme to predict whether species are invasive or non-invasive, as determined by expert opinion for 40 South American aquatic plants in each of 16 regions (*black line*) and for South America as a single continental unit (*red line*). Bootstrapping was used to calculate the confidence intervals. *Upper* and *lower* bands representing 95% level of confidence and the horizontal light grey shape corresponds to the pAUC region. The *diagonal line* represents an area of 0.5 (i.e. complete inability to distinguish between invasive and non-invasive species)

different position to that of Gordon et al. (2012) who used three a priori status, i.e. non-invasive, minor invasive and major invasive. Our results indicate that the USAqWRA scheme is a reliable method to distinguish between non-invasive and invasive aquatic plant species in South America both at region level and at continental scale. The areas under the ROC curves for the 40 South American aquatic plant species assessed were, respectively, equal to 0.853 for the 16 regions (644 assessments) and to 0.893 for South America (153 assessments). Similarly, Conser et al. (2015) tested the Plant Risk Evaluation (PRE) tool by screening 56 known invasive plants and 36 known non-invasive plants and they found a high degree of accuracy for correctly categorising plant species as either high or low risk of invasiveness. On the other hand, Nishida et al. (2009) and McClay et al. (2010) evaluated the Australian Weed Risk Assessment (A-WRA), respectively, for Japan and Canada. Area under the ROC curve was 0.88 for Japan. Areas under the ROC curves for Canada were 0.867 when minor weeds were included as positives (minor weeds were counted as weeds), and 0.845 when only major weeds were counted as positives (only major weeds were considered weeds). Gordon et al. (2012) reported an AUC = 0.96 (when minor invaders were grouped with non-invaders) and AUC = 0.88 (when minor invaders were grouped with major invaders).

The A-WRA scheme is considered effective for different regions across the globe (Pheloung et al., 1999; Gordon et al., 2008; Nishida et al., 2009). However, the cut-off levels have to be selected case by case (Nishida et al., 2009). Gordon et al. (2012) tested USAqWRA for USA using adequate cut-off levels for that region. As remarked by Nishida et al. (2009), different cut-off levels might be required for different study areas. Accordingly, we used different cut-off levels to evaluate the performance of USAqWRA, respectively, for the group of 16 regions (644 assessments) and for South America (153 assessments) as a single continental unit.

The binary classification deriving from USAqWRA is in line with local expert opinion about invasion categories, but it highlights potential invasive species or emergent invaders that are not yet perceived as such by local expert and stakeholders. This could certainly help improve both prevention and early warning strategies. For example, there were 11 species that are not currently

Table 3 The thirty-eight questions of the USAqWRA scheme and their statistical predictability in separating known invasive and non-invasive alien species

Question (Q)#	Q–USAqWRA	FLR	% Q was answered for invasive plants	% Q was answered for non-invasive plants
1	Temperature tolerance	$P = 0.427$	100	100
2	Range of habitat	$P = \mathbf{0.0005}$**	100	100
3	Water/substrate type tolerance	$P = 1.000$	100	100
4	Water clarity tolerance	$P = \mathbf{0.014}$*	100	100
5	Salinity tolerance	$P = 0.066$	93	100
6	pH tolerance	$P = \mathbf{0.046}$*	100	96
7	Water level fluctuation—Tolerates periodic flooding/drying	$P = 0.668$	100	100
8	Lentic—rivers, streams, drains, or other flowing waters, including their margins	$P = 0.126$	100	100
9	Ponds, lakes and other standing waters, including their margins	$P = 1.000$	100	100
10	Swamp, marsh, bog	$P = 0.249$	100	100
11	Establishment—into existing vegetation	$P = 0.074$	93	100
12	Establishment—into disturbed vegetation	$P = 0.167$	100	100
13	Competition—between growth form	$P = 0.082$	100	96
14	Dispersal outside catchment by natural agents (e.g., birds, wind)	$P = 0.170$	100	100
15	Dispersal outside catchment by accidental human activity	$P = 0.379$	93	88
16	Dispersal outside catchment by deliberate introduction	$P = 0.127$	100	96
17	Effective spread within water body/catchment	$P = 0.477$	100	96
18	Generation time (time between germination of an individual and the production of living offspring, not seeds or other dormant structures)	$P = 0.060$	100	100
19	Seeding ability—Quantity	$P = 0.154$	100	100
20	Seeding ability—Viability/persistence	$P = \mathbf{0.045}$*	100	92
21	Vegetative reproduction (Cloning ability)	$P = 0.343$	100	100
22	Obstruction-Physical-water use, recreation	$P = 0.387$	93	100
23	Obstruction-Physical—access	$P = 0.435$	100	100
24	Obstruction- Physical—water flow, power generation	$P = \mathbf{0.025}$*	100	96
25	Obstruction-Physical—irrigation, flood control	$P = \mathbf{0.0002}$**	93	100
26	Aesthetic—visual, olfactory	$P = \mathbf{0.014}$*	100	100
27	Damage to natural areas—Reduces biodiversity	$P = 0.690$	93	96
28	Damage to natural areas—Reduces water quality	$P = 1.000$	100	100
29	Damage to natural areas—Negatively affect physical processes	$P = \mathbf{0.050}$	93	100
30	Human health impairment (e.g., drowning, poisonous, mosquito habitat)	$P = 0.250$	100	100
31	Weed of agriculture, including crops, livestock and aquaculture	$P = 0.075$	100	100
32	Resistance to management—Management—Ease of management implementation	$P = 0.109$	100	96
33	Resistance to management—Management—Recognition of management problem	$P = 0.055$	100	96
34	Resistance to management—Management—Scope of control methods	$P = 0.063$	93	88

Table 3 continued

Question (Q)#	Q–USAqWRA	FLR	% Q was answered for invasive plants	% Q was answered for non-invasive plants
35	Resistance to management—Management—Control method suitability	$P = 0.068$	93	76
36	Resistance to management—Management—Effectiveness of control	$P = 0.158$	93	84
37	Resistance to management—Management—Duration of control	$P = 1.000$	93	76
38	Problem in other countries	$P = 1.000$	100	96

Firth's bias reduced logistic regression (FLR) was used to compare invasive aquatic species with non-invasive aquatic species for each question of the scheme. The significant *P* values (0 '***' 0.001 '**' 0.01 '*' 0.05 '.') are in bold letters. The percentage of time each question (% Q) was effectively answered is also reported

considered invasive by the expert opinion, but were scored as invasive by the USAqWRA, having scores higher than the threshold in at least one region of South America. This disagreement could be related to a general lack of information for South America or awareness of the negative impacts of those species or on their alien status as in the cases of *M. quitense* Kunth (28–45), *Hydrocotyle leucocephala* Cham. & Schltdl., (34–47), *Lemna gibba* L., (36–46), *Lemna minor* L., (40–49), *Salvinia auriculata* Aubl., (44–55), *Eichhornia azurea* (Sw.) Kunth (49–61), *Salvinia molesta* D.S. Mitch., (49–64), *Alternanthera philoxeroides* (Mart.) Griseb., (51–63), *Ludwigia grandiflora* (51-68), *Myriophyllum aquaticum* (Vell.) Verdc. (53-63) and *Pistia stratiotes* (54–67). These species may become invasive in the future, as forecasted by their USAqWRA scores, and also as they are very well known invaders worldwide, as in the case of *Salvinia molesta* and *Pistia stratiotes*. In addition, in many cases, the climatic similarity between the native range and the introduced range might successfully predict establishment and invasiveness risk (Hayes & Barry, 2008; Kumschick & Richardson, 2013).

Importantly, one of these 11 species, i.e. *Ludwigia grandiflora*, is recorded as an invasive alien in many countries outside its native range (Gordon & Gantz, 2011; EPPO, 2015) and it may require proactive management preventing its introduction in the 7 South American regions where it is considered absent.

On the contrary, *Ceratophyllum demersum* (28–39), *Nymphaea alba* (30) and *Typha angustifolia* (39) were considered invasive by the expert opinion, but scored lower with USAqWRA, which suggests that their actual invasive potential requires further attention.

We can highlight that species such as *Spirodela punctata* (G.Mey.) Les & D.J.Crawford (24), *Alisma plantago-aquatica* L. (32), and *Hydrocleys nymphoides* (Humb. & Bonpl. ex Willd.) Buchenau (30–37) (non-invasive according to expert opinion), with scores close to the threshold, may become invasive in the future. In fact, they are ranked as invasive according to Gordon & Gantz (2011) and Gordon et al., 2012 in North America. Those alien species could be considered "sleeping weeds" (Groves, 2006), and they may behave as minor invaders for decades before they become serious invaders.

When evaluating the predictive power of the 56 questions of the final PRE tool, Conser et al. (2015) detected that only 11 of them showed statistical significance in separating invasive from non-invasive species. Four were the result of merging two similar questions, where both were significant or near significant (e.g., different methods of vegetative reproduction, various biotic and abiotic propagule dispersal mechanisms). Similarly to Conser et al. (2015), we evaluated the predictive power of the 38 questions of the USAqWRA scheme for each region and for each species, demonstrating that 8 of them are the most powerful in separating invasive from non-invasive species. These questions were classified into groups because was the result of merging similar questions: (1) Ecology and habitat of the species (questions 2, 4, 6); (2) seeding ability (question 20) and (3) potential of impact and damage to natural areas (questions 24–26, 29).

The application of the USAqWRA to South America resulted in having a specificity equal to 87.5% and higher than the sensitivity (75.75%), with an overall accuracy of 84.9%. This means that the method performs slightly better in identifying non-invasive species than invasive ones; therefore, some invasive species may be undetected (false negatives) while those scored as non-invasive could be considered relatively safe, with lower uncertainty. The application of the method to that investigated 40 species would result in rejecting 37.5% and accepting 62.5% of them if used a pre-border assessment.

Finally, the USAqWRA does not have questions that could specifically take into account the possible modification of the risk assessment outcomes in relation to global change, although several questions do consider the plasticity of the species to varying environmental and site conditions (e.g., temperature).

An increasing number of studies have documented evolutionary changes in invasive populations, typically over ecological timescales (Chown et al., 2015). Additionally, it is generally agreed that over the past century, the potential for aquatic species to expand their ranges at the global level has been enhanced both as a result of the construction of new canals and because of increased international trade (Seebens et al., 2015) and as results of global change and modified socio-economic frameworks (e.g., van Kleunen et al., 2015). Further, the USAqWRA does not include all the modules of a full standard Pest Risk Analysis scheme such as the IPPC/EPPO PRA and does not fulfil many of the minimum criteria for risk assessment of the European Regulation n. 1143/2014 (Roy et al., 2014).

Conclusions

We assessed 40 South American aquatic plant species using the USAqWRA scheme, ranking 17 of them as alien naturalised, and 15 as alien invasive species in at least one region. It is well known that the accuracy of any risk assessment and risk analysis schemes would be benchmarked and compared using test data from very well known species that have been satisfactorily investigated for their biological traits and impacts on biodiversity and related ecosystem services. However, such comprehensive data do not exist for South America.

Furthermore, comprehensive risk estimates are difficult due to various sources of uncertainty (e.g., Dahlstrom et al., 2012). This uncertainty is an inherent component and can stem from a variety of factors, including knowledge gaps and systematic and random measurement error. While expert opinion is often the most appropriate method to make risk estimates under conditions of uncertainty (Halpern et al., 2007) and in a relatively short amount of time, this judgment should be preferably combined with empirical evidence (Dahlstrom et al., 2012) and standard protocols. The available risk classifications from other countries or regions can also be used to help in predicting whether or not a non-native species may become invasive (Verbrugge et al., 2012).

Due the continuously increasing number of non-native species introduction in South America, there is urgent need to adopt and apply prioritisation and express risk assessment schemes which can help identify which new species to the region(s) have the higher potential to become invasive and list those risky aquatic species that can cause ecological negative impacts in South America to prevent attempts of introduction. We consider that our results support the use of the USAqWRA as a screening protocol for South American alien aquatic plant species, providing a rapid assessment scheme that may help reduce the costs of control in the future, and the prioritisation of the species according to their USAqWRA scores,

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