



Risk screening of the potential invasiveness of non-native aquatic species in Vietnam

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Abstract The impact of non-native species, together with their pervasiveness, necessitates a means of identifying which species are most likely to pose an elevated risk of becoming invasive. This is amongst the first applications of the Aquatic Species Invasiveness Screening Kit (AS-ISK) decision support tool in Southeast Asia, and specifically for Vietnam. In total, 30 extant and horizon non-native aquatic species were screened for their potential invasiveness in Vietnam. A threshold score of 6.75 was identified for distinguishing between species

likely to pose a high and low-to-medium risk of becoming invasive in Vietnam for both the basic risk assessment (BRA) and the climate change assessment (BRA + CCA) components of the screening process. However, the absence of nationally-consistent predictions on climate change impacts in Vietnam restricted the applicability of the climate change assessment component. Based on the BRA threshold, of the 30 species screened, 25 were classified as high risk, four as medium risk, and one as low risk. For the BRA, the highest-scoring species were *Hypostomus plecostomus*, *Pomacea canaliculata* and *Lithobates catesbeianus*. This study reliably identified and discriminated between non-invasive and potentially invasive aquatic species in Vietnam, thereby

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providing appropriate AS-ISK score thresholds with which to guide policy and decision-making. This suggests that the AS-ISK could be successfully employed to screen non-native aquatic species in other parts of Southeast Asia.

Keywords AS-ISK · Extant species · Horizon species · Risk analysis · Receiver operating characteristic curve analysis · Viet Nam

Introduction

Although there is increase in knowledge and understanding of the adverse impacts exerted by invasive non-native species in some parts of the world, there remains a strong geographical skew in research effort, with Africa and Asia both seriously understudied in terms of invasion ecology (Pyšek et al. 2008). Exceptions do exist for these continents, such as South Africa (e.g. Ellender and Weyl 2014; Weyl et al. 2016), China (e.g. Wong et al. 2009; Li et al. 2017; Wan et al. 2017) and Japan (e.g. Fausch et al. 2002; Onikura et al. 2011; Matsuzaki and Kadoya 2015). Nonetheless, the disparity in studies of non-native species can be largely explained by differing amounts of financial resources being available in different regions of the world, and in different countries within regions; this, in turn, translates into research intensity (Pyšek et al. 2006). To achieve a more geographically-balanced picture of biological invasions, researchers have called for international cooperation (Pyšek et al. 2008). However, the continuation of such regional geographical bias was recently confirmed, with only 6% of conservation science studies published in 2011–2015 being in Southeast Asia (Di Marco et al. 2017). In the present article, non-native species are defined as organisms that were introduced directly or indirectly by people outside of their natural range of distribution. Invasive non-native species are the subset of non-native species that have become established and dispersed, generating an impact on local ecosystems and species (IPBES 2019).

This relative dearth of studies is even more concerning given that the tropics contain a disproportionately high amount of the world's biological diversity, encompassing more than three-quarters of

all terrestrial, freshwater and marine species (Barlow et al. 2018). Southeast Asia, which comprises the countries of Brunei, Cambodia, Indonesia, Laos, Malaysia, Myanmar (Burma), the Philippines, Singapore, Timor-Leste, Thailand and Vietnam, is a region of exceptionally high species endemism. It is also a region of species endangerment, with the highest proportion of threatened vascular plant, reptile and mammal species when compared to other tropical regions (Sodhi et al. 2010). Indeed, Southeast Asia has been identified as the region of a looming biodiversity disaster. Drivers of biodiversity threat in the region include forest conversion and forest fires, hunting, and wildlife trade (Sodhi et al. 2004). It has previously been postulated that various abiotic and biotic factors (e.g. high species diversity) will minimise the probability of successful establishment of non-native species in undisturbed communities in the tropics (Rejmánek 1996), thus reducing the risk of impacts from invasive species. However, the scale of human disturbance and synergistic impacts that is occurring in Southeast Asia will likely undermine any such mitigation factor (Peh 2010). Indeed, tropical ecosystems are predicted to become increasingly vulnerable to biological invasion as the twenty-first century progresses (Early et al. 2016). Unfortunately, the paucity of research in Southeast Asia (Sodhi et al. 2004, 2010), including on the level and types of impacts caused by invasive non-native species (Nghiem et al. 2013), makes it difficult to establish the relative importance and long-term implications of the latter relative to other threats. As such, the situation with invasive non-native species in Southeast Asia remains very poorly documented and understood (Peh 2010; Nghiem et al. 2013).

Vietnam readily exemplifies this situation, with study of non-native species in the country nascent (Zworykin and Budaev 2013). Thus, the full extent of the establishment of invasive non-native species in Vietnam is currently unknown. For example, 956 non-native plant species (accounting for 9% of the country's known flora) have been reported (Triet et al. 2008 in Le and Truong 2016), but the situation is likely to have changed since 2008. Similarly, for fishes, preliminary and now relatively dated reports indicate that at least nine species have naturalised in the River Mekong basin alone (Welcomme and Vidthayanom 2003; Cacot and Lazard 2009 in Zworykin and Budaev 2013), but there is no national

compendium. The limited published literature that exists tends to focus on species that may affect agriculture (e.g. Dao et al. 2018; Wyckhuys et al. 2019) or human health (e.g. Shan et al. 2018). Surveys for, and/or management of, non-native species that may affect biodiversity are generally limited to protected areas (e.g. Tan et al. 2012; Le and Truong 2016).

In terms of government-led policy and management in Vietnam, a few laws (e.g. Law on Environmental Protection 2014; Law on Biodiversity 2008) make brief reference to non-native species. More specifically, regulation of non-native species that may impact on biodiversity is provided through two lists issued by the Ministry of Natural Resources and Environment. The first list covers ‘invasive’ non-native species (i.e. those that are already present in Vietnam, where they are known to have a substantial negative impact on biodiversity), while the second list is of ‘potentially invasive’ non-native species (i.e. those that are known to have impact on biodiversity in other countries, but have not yet had a significant impact in Vietnam). Separately, the Ministry of Agriculture and Rural Development has issued two lists of species (covering native and non-native species) that are considered capable of causing significant damage to agricultural plants: (1) ‘plant quarantine list I’ is of species (mainly insects, pathogens and plants) that are not yet distributed in Vietnam; and (2) ‘plant quarantine list II’ is of species that currently have a limited distribution in Vietnam. All four lists were developed based on criteria determined by the respective ministries; furthermore, various combinations of terminology, definitions and criteria are used across the four lists, as well as in other legal documents. As with other non-English speaking countries, the situation is further complicated by complexities relating to language. This is because definitions and criteria become less clear, due to increased linguistic uncertainty (Lu 2019; McGeoch et al. 2012), when legal documents, publications or research are translated into English.

The aim of the present study was to identify which extant and potential future (horizon) non-native aquatic species are likely to pose an elevated risk of becoming invasive in Vietnam. The specific objectives were to: (1) carry out screenings using a recently-developed, multilingual taxon-generic

decision-support tool; (2) undertake a calibration in order to identify the threshold between non-native species that are likely to pose a high risk of becoming invasive, and those of low-to-medium risk for Vietnam; and (3) interpret the outcomes within a management context.

Materials and methods

Study area

The risk assessment area, Vietnam, spans latitudes 8°27′ to 23°23′ N and longitudes 102°08′ to 109°30′ E. The country extends 1,662 km from north to south and has a land area of $\approx 331,051 \text{ km}^2$. There are also ≈ 1 million km^2 of sea waters and $\approx 3,000$ islands. If only perennial rivers and streams over 10 km are included, then Vietnam possesses $\approx 2,360$ of them, with a mean density of 0.6 km/km^2 . Although there are nine major river systems in the country, two (the rivers Mekong and Red) are particularly important socio-economically (Ministry of Natural Resources and Environment 2010). As of 2018, the country’s population was over 95 million people, with annual population growth of 1% (World Bank Group 2019).

Vietnam’s climate is tropical, with rainy seasons that correspond to monsoon circulations. Mean annual rainfall ranges from 1400 to 2400 mm and each year the country is affected by multiple typhoons. Annual mean temperature ranges from 12.8 to 27.7 °C (Ministry of Natural Resources and Environment 2010). Projections of future climate conditions in the study region for 2050 indicate: (1) an increase in mean temperatures (ranging from 1.6 to 2.8 °C in different climate zones); (2) increased intensity of extreme weather events, including droughts, typhoons and floods; (3) increases in annual rainfall across all regions, with more extreme precipitation variability between the dry and rainy season; and (iv) a rise of 28–33 cm in sea levels (McElwee 2010; Ministry of Natural Resources and Environment 2009, 2010). However, different regions in the country are likely to have unique climate impacts, making a single national prediction difficult. Overall though, Vietnam is likely to be one of the most significantly impacted nations in the world with respect to climate change due to its long coastline, high dependence on agriculture, and relatively low

levels of development in rural areas (McElwee 2010). Also, the River Mekong is recognised as one of the world's most vulnerable mega-deltas, especially as a result of climate change-induced sea-level rise (Whitehead et al. 2019).

Toolkit description

Risk screenings were undertaken using the Aquatic Species Invasiveness Screening Kit (AS-ISK), which is available for free download at www.cefas.co.uk/nns/tools/ (Copp et al. 2016a). The AS-ISK can be used to screen 27 groups of aquatic organisms for freshwater, brackish and marine habitats and, since release of version 2.1, the AS-ISK has been available in 32 languages, including Vietnamese (Copp et al. 2021). The AS-ISK was constructed within the architecture of the Fish Invasive Screening Kit v2 (Lawson et al. 2013) using questions from the generic screening module of the European Non-native Species in Aquaculture Risk Analysis Scheme (Copp et al. 2016b). These questions were retained and/or revised, with additional questions and features, to comply with the 'minimum standards' (Roy et al. 2018) for the assessment of non-native species, as per the recent EC 'Regulation No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species'. The AS-ISK consists of 55 questions: the first 49 cover the bio-geographical and biological aspects of the species under assessment and comprise the Basic Risk Assessment (BRA). The remaining six questions require the assessor to predict how future climatic conditions are likely to affect the BRA with respect to risks of introduction, establishment, dispersal and impact, and these comprise the Climate Change Assessment (CCA).

To achieve a valid AS-ISK assessment, the assessor must provide a response, justification and level of confidence to each question, with the result being a BRA score (ranging from -20 to 68) and a composite BRA + CCA score (ranging from -32 to 80). Basic AS-ISK scores <1 suggest that the species is unlikely (i.e. poses a low risk) to become invasive in the risk assessment area, whereas scores >1 indicate a medium-to-high risk of the species becoming invasive in the risk assessment area. The threshold BRA and BRA + CCA values that distinguish between medium and high risk levels

are typically obtained through a procedure of 'calibration' that is specific to the risk assessment area, which is undertaken by Receiver Operating Characteristic (ROC) curve analysis (Bewick et al. 2004) (see *Statistical analysis*).

In the AS-ISK, confidence in responses to questions (Qs) are ranked using a 1–4 scale (1 = low; 2 = medium; 3 = high; 4 = very high), as per the Intergovernmental Panel on Climate Change (IPCC 2005; Copp et al. 2016a). Based on the confidence level (CL) allocated to each response, a confidence factor (CF) is obtained as:

$$CF = \sum (CL_{Q_i}) / (4 \times 55) \quad (i = 1, \dots, 55)$$

where CL_{Q_i} is the CL for Q_i , 4 is the maximum achievable value for confidence (i.e. very high; see above) and 55 is the total number of Qs in the AS-ISK. Based on the BRA (49 Qs) and the CCA (6 Qs), the CL_{BRA} and CL_{CCA} are also computed. The CF ranges from a minimum of 0.25 (i.e. all 55 Qs with confidence level equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Two additional CFs can be computed; namely, the CF_{BRA} and the CF_{CCA} (hence, similar to the CL).

Species selection and data processing

In total, 30 non-native aquatic species were screened for their potential invasiveness in the risk assessment area. Species were selected according to the following criteria: (1) extant; that is, already present in the risk assessment area ($n = 26$), and (2) horizon; that is, not yet reported but likely to enter the risk assessment area in the future ($n = 4$) (Table 1). Screenings were carried out by the first three authors (LR, KATT, TDB), who are knowledgeable in the biology and ecology of the aquatic species of the region, with GHC and LV being responsible for overseeing construction of the species list and for quality control of the generated AS-ISK database of screenings, respectively.

Following computation of the BRA and BRA + CCA scores, ROC curve analysis was used to assess the predictive ability of the AS-ISK to discriminate between species posing a high risk and those posing a medium or low risk of being invasive for the risk assessment area. For ROC curve analysis to be implemented, species need to be categorised a

Table 1 Extant and horizon non-native aquatic species (with taxonomy) screened for their risk of invasiveness in Vietnam with the Aquatic Species Invasiveness Screening Kit (AS-ISK)

Criterion/group/phylum	Class	Order	Family	Taxon name	Common name	
<i>Extant</i>						
Reptiles						
Chordata	Reptilia	Crocodylia	Crocodylidae	<i>Crocodylus rhombifer</i>	Cuban crocodile	
		Testudines	Emydidae	<i>Trachemys scripta elegans</i>	Red-eared slider	
Amphibians						
Chordata	Amphibia	Anura	Bufonidae	<i>Rhinella marina</i>	Cane toad	
			Dicroglossidae	<i>Hoplobatrachus tigerinus</i>	Indus valley bullfrog	
Freshwater fishes						
Chordata	Chondrostei	Acipenseriformes	Acipenseridae	<i>Acipenser baerii</i>	Siberian sturgeon	
Chordata	Teleostei	Characiformes	Prochilodontidae	<i>Prochilodus lineatus</i>	Streaked prochilod	
			Serrasalminidae	<i>Piaractus brachypomus</i>	Pirapitinga	
			Cypriniformes	Cyprinidae	<i>Cirrhinus cirrhosus</i>	Mrigal carp
		Cyprinodontiformes	Perciformes	Cichlidae	<i>Labeo rohita</i>	Roho labeo
					<i>Gambusia affinis</i>	Mosquitofish
					<i>Cichla ocellaris</i>	Peacock cichlid
					<i>Oreochromis mossambicus</i>	Mozambique tilapia
		Salmoniformes	Siluriformes	Salmonidae	<i>Oreochromis niloticus</i>	Nile tilapia
					<i>Oncorhynchus mykiss</i>	Rainbow trout
					Clariidae	<i>Clarias gariepinus</i>
Ictaluridae	<i>Ictalurus punctatus</i>	Channel catfish				
	Loricariidae	<i>Hypostomus plecostomus</i>	Suckermouth catfish			
			<i>Pterygoplichthys pardalis</i>	Amazon sailfin catfish		
Freshwater invertebrates						
Arthropoda	Malacostraca	Decapoda	Cambaridae	<i>Procambarus clarkii</i>	Red swamp crayfish	
Mollusca	Gastropoda	Architaenioglossa	Ampullariidae	<i>Pomacea canaliculata</i>	Channeled applesnail	
				<i>Pomacea diffusa</i>	Spiketop applesnail	
Brackish invertebrates						
Arthropoda	Malacostraca	Decapoda	Penaeidae	<i>Penaeus vannamei</i>	Whiteleg shrimp	
Marine invertebrates						
Arthropoda	Malacostraca	Decapoda	Parastacidae	<i>Cherax quadricarinatus</i>	–	
Mollusca	Bivalvia	Ostreoida	Ostreidae	<i>Crassostrea gigas</i>	Giant cupped oyster	
Freshwater plants						
Tracheophyta	Magnoliopsida	Nymphaeales	Cabombaceae	<i>Cabomba caroliniana</i>	–	
Marine plants						
Rhodophyta	Florideophyceae	Gigartinales	Solieriaceae	<i>Kappaphycus alvarezii</i>	Elkhorn sea moss	
<i>Horizon</i>						
Amphibians						
Chordata	Amphibia	Anura	Ranidae	<i>Lithobates catesbeianus</i>	American bullfrog	
Freshwater fishes						

Table 1 continued

Criterion/group/phylum	Class	Order	Family	Taxon name	Common name
Chordata	Teleostei	Characiformes	Serrasalimidae	<i>Pygocentrus nattereri</i>	Red piranha
		Perciformes	Centrarchidae	<i>Micropterus dolomieu</i>	Smallmouth bass
				<i>Micropterus salmoides</i>	Largemouth bass

Groups are according to the aquatic organism categorisation used in AS-ISK

priori in terms of their documented invasiveness (i.e. non-invasive or invasive). In general, a priori categorisation of fishes is facilitated by the availability of online databases (i.e. FishBase). However, owing to more limited (literature-based) information being available for other groups of organisms, an ‘integrated approach’ was adopted in the present study to determine the a priori invasiveness status of species other than fishes: (1) similar to previous AS-ISK applications to fishes (i.e. Glamuzina et al. 2017; Li et al. 2017; Tarkan et al. 2017a, b; Bilge et al. 2019; Dodd et al. 2019; Clarke et al. 2020; Interesova et al. 2020; Uyan et al. 2020), a first search was made of FishBase (www.fishbase.org) and, for invertebrates, of SeaLifeBase (www.sealifebase.org), with the species categorised a priori as invasive if it was listed as a ‘potential pest’ and as non-invasive if it was listed as ‘harmless’; (2) a second search was made of the Global Invasive Species Database (GISD: www.iucngisd.org), with the species categorised a priori as invasive if it was listed therein; (3) a third search was made of the Invasive Species of Japan database (www.nies.go.jp/biodiversity/invasive/index_en.html) and the Invasive and Exotic Species of North America list (www.invasive.org), with the species categorised a priori as ‘invasive’ if listed therein; (4) if the species was absent from any of the previous databases, then a Google Scholar (literature) search was performed (using the keywords ‘invasive’, ‘invasiveness’ and ‘impact’ along with that of the species) to check whether at least one peer-reviewed reference in support was found. The latter was then taken as ‘sufficient evidence’ for categorising the species a priori as invasive; whereas, if no evidence was found, then the species was categorised a priori as non-invasive. Notably, if a species was listed as harmless in FishBase but found to be invasive in any of the other steps of the process, then the a priori categorisation of the species became that of invasive.

Statistical analysis

A ROC curve is a graph of sensitivity versus 1 – specificity (or alternatively, sensitivity vs. specificity) for each threshold value where, in the present context, sensitivity and specificity will be the proportion of a priori invasive and non-invasive aquatic species that are correctly identified by the AS-ISK as such. A measure of the accuracy of the calibration analysis is the Area Under the Curve (AUC), which typically ranges from 0.5 to 1; the closer to 1, the better the ability to differentiate between invasive and non-invasive species. If the AUC is equal to 1 then the test is 100% accurate because both sensitivity and specificity are 1, and there are neither ‘false positives’ (a priori non-invasive species classified as high risk, hence invasive) nor ‘false negatives’ (a priori invasive species classified as low risk, hence non-invasive). Conversely, if the AUC is equal to 0.5, then the test is 0% accurate as it cannot discriminate between ‘true positives’ (a priori invasive species classified as high risk, hence invasive) and ‘true negatives’ (a priori non-invasive species classified as low risk, hence non-invasive). AUC values are generally interpreted as follows: $0.7 \leq \text{AUC} < 0.8$ = acceptable discriminatory power, $0.8 \leq \text{AUC} < 0.9$ = excellent, $0.9 \leq \text{AUC}$ = outstanding (Hosmer et al. 2013). Following ROC curve analysis, the best AS-ISK threshold value that maximises the true positives rate and minimises the false positives rate was determined using Youden’s *J* statistic; whereas, a ‘default’ threshold of 1 was set to distinguish between low- and medium-risk species (see *Toolkit description*). ROC curve analysis was carried out with the package ‘pROC’ (Robin et al. 2011) for R x64 v3.2.0 (R Development Core Team 2020) using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed along the entire range of sensitivity points (i.e. 0–1, at 0.1 intervals).

Differences between mean confidence level and mean confidence factor for the BRA (CL_{BRA} and CF_{BRA} , respectively) and for the CCA (CL_{CCA} and CF_{CCA} , respectively) were tested by permutational ANOVA based on a one-factor design (i.e. Component, with two levels: BRA and CCA). Analysis was carried out in PERMANOVA+ for PRIMER v6, with normalisation of the data and using a Bray-Curtis dissimilarity measure, 9999 unrestricted permutations of the raw data (Anderson et al. 2008), and with statistical effects evaluated at $\alpha = 0.05$.

Results

As ROC curve analysis yielded the same BRA and BRA + CCA thresholds when applied to all aquatic species and to fishes only, the outcomes for all aquatic species are reported here. The ROC curve for the BRA resulted in an AUC of 0.8333 (0.5067–1.0000 95% CI) and that for the BRA + CCA in an AUC of 0.8160 (0.5223–1.0000 95% CI). These AUCs were >0.5 and had excellent discriminatory power, indicating that the AS-ISK was able to discriminate reliably between non-invasive and invasive non-native species for the risk assessment area. Youden's J provided the same threshold of 6.75 for both the BRA and the BRA + CCA; this was used for calibration of the risk outcomes. Accordingly, the BRA threshold allowed distinction between medium-risk species, with scores within the interval [1, 6.75[, and high-risk species, with scores within]6.75, 68]. The BRA + CCA threshold allowed distinction between medium-risk species, with scores within [1.0, 6.75[, and high-risk species, with scores within]6.75, 80]. In contrast, species classified as low risk were those with BRA scores within [-20, 1[and BRA + CCA scores within [-32, 1[. Note that thresholds reported hereafter are presented using the appropriate statistical use of interval brackets: '[' and '[' (www.mathwords.com/i/interval_notation.htm).

Of the 30 species screened (see Supplementary Material for AS-ISK reports), based on the BRA threshold, 25 (83.3%) were classified as high risk, four (13.3%) as medium risk, and one (3.3%) as low risk (Table 2). All 22 species categorised a priori as

invasive were true positives, and amongst the eight species categorised a priori as non-invasive, one was a true negative (Cuban crocodile *Crocodylus rhombifer*) and three were false positives (suckermouth catfish *Hypostomus plecostomus*, elkhorn sea moss *Kappaphycus alvarezii* and whiteleg shrimp *Penaeus vannamei*). All four medium-risk species were categorised a priori as non-invasive. Based on the BRA + CCA threshold, 23 (76.7%) species were classified as high risk, four (13.3%) as medium risk, and three (10.0%) as low risk. Of the 22 species categorised a priori as invasive, 20 were true positives and there was one false negative (pirapitinga *Piaractus brachypomus*); amongst the eight species categorised a priori as non-invasive, there were two true negatives (*Crocodylus rhombifer* and streaked prochilod *Prochilodus lineatus*) and the same three false positives as for the BRA. Of the four medium-risk species, three were categorised a priori as non-invasive and one as invasive (rainbow trout *Oncorhynchus mykiss*).

For the BRA, the highest-scoring species (score ≥ 40 , taken as an ad hoc 'very high risk' threshold) were suckermouth catfish *Hypostomus plecostomus*, channelled apple snail *Pomacea canaliculata* and American bullfrog *Lithobates catesbeianus* (from higher to lower scores). For the BRA + CCA, the highest-scoring species included all the above plus cane toad *Rhinella marina*. For both the BRA and the BRA + CCA, Siberian sturgeon *Acipenser baerii*, Mrigal carp *Cirrhinus cirrhosus* and Roho labeo *Labeo rohita* were classified as medium risk, and *Crocodylus rhombifer* as low risk (Table 2). The CCA resulted in an increase relative to the BRA score for five (16.7%) species, in a decrease for four (13.3%), and in no change for the remaining 21. Notably, Nile tilapia *Oreochromis mossambicus* and *Rhinella marina* achieved the largest possible positive change in score of 12, and *Piaractus brachypomus* and streaked prochilod *Prochilodus lineatus* the largest possible negative change in score of -12 (Table 2).

The mean CL (i.e. over all 55 questions) was 2.92 ± 0.06 SE, the mean CL_{BRA} 3.00 ± 0.06 SE, and the mean CL_{CCA} 2.26 ± 0.14 SE (hence, in all cases indicating medium to high confidence). The CL_{BRA} was significantly higher than the CL_{CCA} ($F_{1,58}^{\#} = 25.56, P < 0.001$). Similarly, mean values for

Table 2 Non-native aquatic species screened with AS-ISK for Vietnam

Species name	Assessor	A priori categorisation	Component				Delta	Confidence					
			BRA		BRA + CCA			CL			CF		
			Score	Outcome	Score	Outcome		Total	BRA	CCA	Total	BRA	CCA
<i>Acipenser baerii</i>	TDB	N	6.0	Medium	4.0	Medium	-2.0	3.2	3.3	3.0	0.81	0.82	0.75
<i>Cabomba caroliniana</i>	LR	Y	35.0	High	37.0	High	2.0	2.7	2.9	1.0	0.68	0.73	0.25
<i>Cherax quadricarinatus</i>	KATT	Y	18.0	High	18.0	High	0.0	2.5	2.6	2.0	0.63	0.65	0.50
<i>Cichla ocellaris</i>	KATT	Y	19.0	High	19.0	High	0.0	3.1	3.3	2.0	0.79	0.82	0.50
<i>Cirrhinus cirrhosus</i>	TDB	N	6.0	Medium	6.0	Medium	0.0	3.3	3.4	3.0	0.84	0.85	0.75
<i>Clarias gariepinus</i>	KATT	Y	25.0	High	25.0	High	0.0	3.1	3.2	2.2	0.78	0.81	0.54
<i>Crassostrea gigas</i>	TDB	Y	21.0	High	21.0	High	0.0	3.2	3.2	3.2	0.80	0.80	0.79
<i>Crocodylus rhombifer</i>	LR	N	-2.0	Low	-2.0	Low	0.0	2.2	2.3	1.5	0.55	0.57	0.38
<i>Gambusia affinis</i>	KATT	Y	19.0	High	19.0	High	0.0	3.0	3.1	2.0	0.75	0.79	0.50
<i>Hoplobatrachus tigerinus</i>	TDB	Y	15.0	High	15.0	High	0.0	2.9	2.9	3.0	0.74	0.73	0.75
<i>Hypostomus plecostomus</i>	KATT	N	42.0	High	42.0	High	0.0	2.9	3.1	2.0	0.74	0.77	0.50
<i>Ictalurus punctatus</i>	TDB	Y	21.0	High	21.0	High	0.0	3.2	3.2	3.2	0.80	0.80	0.79
<i>Kappaphycus alvarezii</i>	TDB	Y	16.0	High	16.0	High	0.0	3.1	3.2	3.0	0.79	0.79	0.75
<i>Labeo rohita</i>	TDB	N	5.0	Medium	5.0	Medium	0.0	3.3	3.3	3.0	0.83	0.84	0.75
<i>Lithobates catesbeianus</i>	LR	Y	40.0	High	46.0	High	6.0	2.7	2.9	1.0	0.68	0.73	0.25
<i>Micropterus dolomieu</i>	KATT	Y	7.5	High	7.5	High	0.0	2.6	2.7	2.0	0.65	0.66	0.50
<i>Micropterus salmoides</i>	KATT	Y	7.5	High	7.5	High	0.0	2.6	2.6	2.0	0.64	0.66	0.50
<i>Oncorhynchus mykiss</i>	TDB	Y	8.0	High	6.0	Medium	-2.0	3.4	3.4	3.5	0.85	0.85	0.88
<i>Oreochromis mossambicus</i>	KATT	Y	27.0	High	39.0	High	12.0	3.2	3.4	2.2	0.81	0.84	0.54
<i>Oreochromis niloticus</i>	TDB	Y	28.0	High	28.0	High	0.0	3.4	3.4	3.0	0.84	0.85	0.75
<i>Penaeus vannamei</i>	TDB	Y	12.0	High	14.0	High	2.0	3.2	3.2	3.2	0.80	0.81	0.79
<i>Piaractus brachyopomus</i>	KATT	Y	9.5	High	-2.5	Low	-12.0	2.5	2.7	1.2	0.64	0.68	0.29
<i>Pomacea canaliculata</i>	KATT	Y	41.0	High	41.0	High	0.0	2.9	3.0	2.0	0.72	0.74	0.50
<i>Pomacea diffusa</i>	KATT	Y	8.0	High	8.0	High	0.0	2.4	2.4	2.0	0.60	0.61	0.50
<i>Procambarus clarkii</i>	KATT	Y	39.0	High	39.0	High	0.0	2.9	3.1	2.0	0.74	0.77	0.50
<i>Prochilodus lineatus</i>	KATT	N	5.0	Medium	-7.0	Low	-12.0	3.0	3.2	1.2	0.74	0.80	0.29
<i>Pterygoplichthys pardalis</i>	KATT	Y	38.0	High	38.0	High	0.0	2.7	2.8	2.0	0.68	0.70	0.50
<i>Pygocentrus nattereri</i>	KATT	Y	18.0	High	18.0	High	0.0	2.6	2.6	2.0	0.64	0.66	0.50
<i>Rhinella marina</i>	KATT	Y	33.0	High	45.0	High	12.0	3.2	3.2	3.2	0.80	0.80	0.79
<i>Trachemys scripta elegans</i>	KATT	Y	33.0	High	33.0	High	0.0	2.3	2.4	1.3	0.57	0.60	0.33

For each species, the following are provided: assessor (author's initials), a priori categorisation (N=non-invasive; Y=invasive); Basic Risk Assessment (BRA) and BRA plus Climate Change Assessment (BRA + CCA) scores with corresponding risk outcomes; the difference (Delta) between BRA + CCA and BRA scores; Confidence Level (CL) and Confidence Factor (CF) (see text for explanation) for all questions (Total) and separately for the BRA and CCA components of the risk assessment. Risk outcomes are based on a threshold of 6.75 for both the BRA (Low: score within interval [-20, 1]; Medium: [1, 6.75]; High: [6.75, 68]) and the BRA + CCA (Low: [-32, 1]; Medium: [1, 6.75]; High: [6.75, 80]). Note that thresholds reported hereafter are presented using the appropriate statistical use of interval brackets: '[' and ']' (www.mathwords.com/i/interval_notation.htm). Combined AS-ISK report for the screened species in the Supplementary Material

CF = 0.730 ± 0.016 SE and CF_{BRA} = 0.751 ± 0.015 SE were higher than the mean value for the CF_{CCA} = 0.564 ± 0.034 SE, and the mean CF_{BRA} was significantly higher than the mean CF_{CCA} (with the same significance values as for the CL_{BRA} vs. CL_{CCA} comparison, due to the two indices being related).

Discussion

This study represents the first application of the AS-ISK for Vietnam and amongst the first for Southeast Asia in general. As with previous applications in other regions and countries (Glamuzina et al. 2017;

Li et al. 2017; Tarkan et al. 2017a, b; Semenchenko et al. 2018; Bilge et al. 2019; Dodd et al. 2019; Clarke et al. 2020; Killi et al., 2020; Interesova et al. 2020; Uyan et al. 2020), the AS-ISK could reliably discriminate between invasive and non-invasive species in the risk assessment area. Furthermore, it is notable that only one false positive was identified, and that all species categorised a priori as invasive were ROC classified as high-risk (hence, true positives) for the BRA, and the vast majority of them also for the BRA + CCA. This result validates the adoption of the AS-ISK decision-support tool not only for Vietnam but ultimately for Southeast Asia.

The current risk screening for Vietnam established the same threshold of 6.75 for both the BRA and the BRA + CCA. Low threshold values calibrated for other risk assessment areas (i.e. Glamuzina et al. 2017; Semenchenko et al. 2018; Tarkan et al. 2017a) have been attributed to the high number of species translocated within those risk assessment areas, in particular to enclosed, artificial water bodies. The reasons for the low threshold score for Vietnam may be similar, given that Vietnam is a major hub for wildlife trade (Nguyen 2008), wildlife consumption (e.g. Venkataraman 2007), and commercial farming of wildlife (Drury 2009). It also has a culture of keeping both exotic and native animals as pets (e.g. Eaton et al. 2017) and of releasing large quantities of various species into the environment (particularly aquatic species into water bodies) during ceremonies (e.g. *Tết*). These activities, together with a tropical climate and broad latitudinal extent, provide substantial scope for both the introduction and establishment of non-native species in Vietnam. Overall, this suggests that non-native aquatic species, and especially fishes, pose a considerable threat to native species and ecosystems in Vietnam.

In terms of individual species, the highest-scoring species in the present study (i.e. *Hypostomus plecostomus*, *Lithobates catesbeianus*, *Pomacea canaliculata* and *Rhinella marina*) have all been categorised as invasive in the region, nearby regions, or internationally. For example, *P. canaliculata* has been widely introduced in Asia, including Vietnam, and has resulted in extensive damage to both agricultural land and native ecosystems, particularly wetlands (Carlsson et al. 2004; Joshi et al. 2017). Meanwhile, the situation with *L. catesbeianus* and *Rhinella marina* demonstrates the scale of knowledge

gaps for non-native species in Vietnam. Both species are on the list of the ‘World’s Worst 100 Invaders’ and, in the case of *L. catesbeianus*, occurs in at least 41 countries on four continents, including neighbouring/nearby Asian countries e.g. Japan, Thailand, China, Taiwan (www.iucngisd.org/gisd/species.php?sc=80). There are no readily-available records for either species in Vietnam; however, given the proximity of their known non-native distribution, it is feasible that they occur in the country but have not been officially recognised.

Amongst the highest scoring of the screened species, *Hypostomus plecostomus* was the lone false positive. Notably, both the screening and the result for this species, as well as for *Pterygoplichthys pardalis*, are complicated by the fact that there is a high degree of taxonomic uncertainty for loricariids in general, and for species of the genera *Hypostomus* and *Pterygoplichthys* in particular (www.cabi.org/isc/datasheet/114927). Although *Hypostomus* was re-described by Armbruster (2004), additional taxonomic and systematic work is required because it is still difficult to identify most hypostomids to species level; this renders species identifications tentative if not tenuous. There are also no distinctive characteristics with which to diagnose the genus (Armbruster 2004); indeed, historically, *Pterygoplichthys* species have often been misidentified as hypostomids (www.fws.gov/fisheries/ans/erss/uncertainrisk/ERSS-Hypostomus-plecostomus-final.pdf; <http://nas.er.usgs.gov/>). Confounding issues in the present risk screening are: (1) both species have the same (multiple) common names in Vietnamese, and (2) multiple species of the genus can occur in a single location (Hoover et al. 2004). Using available literature, this makes it difficult to attribute with confidence the reported impacts to one of the two species.

The identical threshold score identified in the present study for distinguishing between medium- and high-risk species under current (BRA) and future (BRA + CCA) climate conditions contrasts other applications of the AS-ISK, where an increase in threshold value from BRA to BRA + CCA was identified for the majority of the species screened (i.e. Semenchenko et al. 2018; Bilge et al., 2019; Dodd et al. 2019; Clarke et al. 2020; Killi et al., 2020; Interesova et al. 2020; Uyan et al. 2020). The reason for the discrepancy may be attributed to the relatively

low understanding of, and therefore confidence in, projected climate change impacts in Vietnam. This is further complicated by the fact that, owing to their geographical locations, various regions within Vietnam are likely to be affected differently by future changes. Overall, this makes it difficult to produce a single national projection (McElwee 2010). This climo-geographic issue is likely (if not certainly) to affect any risk screening or full assessment undertaken for any non-native species with respect to the entire country of Vietnam; it is also well demonstrated for AS-ISK screenings for Great Britain (Dodd et al. 2019) and for Italy with regard to channel catfish *Ictalurus punctatus* (Haubrock et al. 2021). This contrasts with other screening applications, which have tended to be for geographically-specific risk assessment areas (e.g. Glamuzina et al. 2017; Bilge et al. 2019), or for a country smaller than Vietnam and one that is therefore more climatically uniform (e.g. Belarus: Semenchenko et al. 2018).

Of note in the present study are the identical thresholds for current (BRA) and future (BRA + CCA) climate conditions when applied to all aquatic species and to fish species only. This means that this threshold can be reliably used to guide management decisions for all the taxonomic groups that were screened in the present study. However, because risk analysis is a dynamic process (Copp et al. 2016a), these risk screenings would need to be updated and revised when climate-change modelling within a climo-geographic context has been undertaken for Vietnam.

Conservation and management considerations

Research has identified that, except for unintentional hitchhiker (e.g. contaminant) species, non-native species are not randomly selected. Rather, humans tend to select species whose attributes are conducive to invasion success; furthermore, these species are introduced to areas in which the co-evolved enemies that limit the species' abundance in their native range are absent (Buckley and Catford 2015; Rejmánek and Simberloff 2017). The main introduction vectors for aquatic non-native species to Vietnam presumably include aquaculture, captive breeding and deliberate release to the wild, as well as the pet/aquarium trade, though ranking of these is currently not possible.

The assessment of existing and potential future species (so-called horizon scanning) is crucial for identifying the most threatening of the potential invasive non-native species that do not yet occur in a risk assessment area (Roy et al. 2019). Failure to use a systematic, evidence-based approach for such assessments can lead to inconsistent and incomparable outcomes (Roy et al. 2018). To address this, the AS-ISK was enhanced to be consistent with the European Union (EU) practice of offering documents in national languages (Copp et al. 2021). Although the AS-ISK does not offer all official languages of EU member states, it does offer many of them, as well as several Asian languages (Chinese, Japanese, Korean, Filipino, Vietnamese, and Thai). Other risk screening tools exist for non-native species, such as Invasive Species Environmental Impact Assessment (Branquart 2009) and the Canadian Marine Invasive Species Tool (Drolet et al. 2016). However, few of the extant decision-support tools for non-native species are offered in more than one or a few languages. The AS-ISK (since v2.1) is the only known decision-support tool that offers the user the option of carrying out their screening in Vietnamese and, as such, serves as a means of communicating risks and uncertainties about non-native species to Vietnamese stakeholders and decision-makers (Copp et al. 2021).

As with its predecessor decision-support tool, the freshwater Fish Invasiveness Screening Kit (FISK: Copp et al. 2009; Vilizzi et al. 2019), the AS-ISK is not intended to make decisions but, rather, to inform policy and decision-makers. It is therefore an aid in the development of non-native species-related legislation, policy, and management strategies. In the context of Vietnam, the AS-ISK application and calibration presented here are intended to act as a guide for identifying which aquatic species are likely to be invasive, and thus to have adverse impacts on native biodiversity, ecosystem function and services. This information can then contribute to the establishment of invasive non-native species 'watch' lists and customs restrictions, as well as prioritisation of which species to control.

In Vietnam, decision-making relating to non-native (potentially) invasive species is the purview of the Ministry of Natural Resources and Environment. However, there is overlap with the Ministry of Agriculture and Rural Development, as invasive non-

native species could impact both biodiversity and agriculture, and the latter ministry has remit to control species that (potentially) impact on crops, forestry, animal husbandry and fisheries. The exploration of the AS-ISK as a potential toolkit for use in Vietnam was driven by recognition by the two ministries that a more systematic approach is needed. It was facilitated though a collaboration between invasive species practitioners in Vietnam and the European developers of the AS-ISK. Initial application was challenged by the then-absence of the toolkit in Vietnamese; however, further collaboration allowed the toolkit (version 2.1 onwards) to be translated to Vietnamese. It is hoped that this functionality, together with the setting of BRA and BRA + CCA thresholds that are specific to Vietnam, will make the AS-ISK accessible to Vietnamese practitioners, researchers, and decision-makers. It is feasible that application of risk-screening toolkits such as the AS-ISK to other developing countries, particularly in Southeast Asia, could follow a similar process.

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