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Identifying invasive fish species threats to RAMSAR wetland sites in the Caspian Sea region—A case study of the Anzali Wetland Complex (Iran)

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Abstract

Risk screening tools play a crucial role in identifying potential high-risk non-native (NN) fish species. In this study, potentially invasive NN fish species in the Anzali Wetland Complex (AWC), which is located on the south coast of the Caspian Sea (Iran), were identified using the Aquatic Species Invasiveness Screening Kit (AS-ISK). Twenty-nine freshwater fish species were screened of which 13 exist in the AWC and 16 in close proximity to it ("horizon" species). Receiver operating characteristic curve analysis showed that AS-ISK could discriminate reliably between non-invasive and invasive fish species for the AWC. Mean threshold scores were 3.25 for the Basic Risk Assessment (BRA) and 11.75 for the BRA + CCA (BRA + Climate Change Assessment), and these, respectively, classified 89.7% and 86.2% of the species as high risk. The CCA resulted in an increase in the BRA scores for 86.2% of the species, suggesting the need to account in future NN species management for a likely increased invasiveness of those species under future climate conditions. These results suggest that AS-ISK could prove an effective tool for identifying potentially invasive NN freshwater fishes in other wetlands of the Caspian Sea basin.

KEYWORDS

alien species, Aquatic Species Invasiveness Screening Kit, horizon species, RAMSAR, risk analysis, risk assessment

1 | INTRODUCTION

In view of the potential adverse impacts that invasive non-native (NN) species can exert on aquatic ecosystems (Cox & Kitchell, 2004; Dudgeon et al., 2006; Pimentel, Zuniga, & Morrison, 2005), the identification of the risks posed by existing and potential future NN species is crucial to inform decisions in wetland management and rehabilitation (D'Antonio & Meyerson, 2002; Rahel & Olden, 2008; Vilizzi, Thwaites, Smith, Nicol, & Madden, 2014). This is of relevance to arid regions, where freshwater wetlands represent an important habitat for both resident and migratory species (e.g. Angeler, Alvarez-Cobelas, Sanchez-Carrillo, & Rodrigo, 2002; Brinson & Malvárez, 2002).

Wetlands associated to the Caspian Sea, which is surrounded by five oil-producing countries (i.e. Azerbaijan, Iran, Kazakhstan, Russia and Turkmenistan), are at risk of bioinvasions by way of ship-related vectors (e.g. ship ballast water, hull fouling) and their associated pathways. Ballast water has the potential to transport a wide range of organisms, including freshwater fishes (e.g.

Grigorovich et al., 2003; Moyle & Light, 1996). Preventing the introduction of potentially invasive species to avoid invaded ecosystems has been considered both in relevant national action plans pertaining to nature conservation, biodiversity and sustainability (e.g. Azerbaijan Government, 2016; Dabiri, Fazel, Moghaddasi, & Mehrdadi, 2016; Turkmenistan Government, 2002) and in international treaties in which Caspian Sea countries are contracting parties (e.g. RAMSAR Convention, International Maritime Organization, Tehran Convention). In this regard, risk analysis of invasive NN species is increasingly mandated by international and national policies and enables improved management of invasive species (Lodge et al., 2016). Indeed, Clarke et al. (2020) identified potential invasive (extant and horizon) fish species to prioritise NN fishes for the targeted monitoring and management in the Arabian/Persian Gulf and Sea of Oman region.

Amongst the Caspian Sea wetlands is the Anzali Wetland Complex (AWC), which is located in the southern Caspian Sea (north Iran) and represents an important Ramsar biodiversity site (Esmaeili, Teimori, Owfi, Abbasi, & Coad, 2014; Jafari, 2009). More than 52 fish species inhabit the AWC (Abbasi et al., 2017) of which 15 are NN (Abbasi et al., 2017; Abdoli & Naderi, 2009), and of the 159 species comprising the Caspian Sea fish fauna (Naseka & Bogutskaya, 2009), 52 (33%) are found in the AWC.

Deliberate introductions of commercial NN fish species to the Caspian Sea began in 1902 as part of acclimation programmes (Leppäkoski et al., 2002), whereas unintentional introductions of some NN fish species occurred by way of natural dispersal via the Volga canal, which acts as an invasion corridor and links the Black and Azov seas to the Caspian Sea (Panov et al., 2009). Vectors for NN fish introductions to the AWC include the following: (a) stocking of Asian carps, that is, grass carp *Ctenopharyngodon idella* (Valenciennes), silver carp *Hypophthalmichthys molitrix* (Valenciennes), bighead carp *Hypophthalmichthys nobilis* (Richardson), as well as golden grey mullet *Chelon auratus* (Risso) and leaping mullet *Chelon saliens* (Risso) (Abbasi et al., 2017; Coad & Abdoli, 1993; Holčík & Oláh, 1992); (b) biological control, for example, eastern mosquitofish *Gambusia holbrooki* Girard; (c) release of unwanted pet fish such as goldfish *Carassius auratus* (Linnaeus) (Coad & Abdoli, 1993); and (d) accidental contaminants of intentionally imported consignments of Asian carp species, namely *Carassius auratus*, sharpbelly *Hemiculter leucisculus* (Basilewsky) and topmouth gudgeon *Pseudorasbora parva* (Temmink & Schlegel) (Abbasi et al., 2017; Coad & Abdoli, 1993). Accidental contamination of Asian carp consignments was the same introduction vector identified for *P. parva* throughout Europe (Gozlan et al., 2010). Pacific salmonids were also introduced into the Caspian Sea from 1962 to 1979, but they did not establish, and the last known specimens of chum salmon *Oncorhynchus keta* (Walbaum) in Iranian waters were recorded in 1989 (Holčík & Razavi, 1992). Also, the AWC is linked to the Caspian Sea and its catchment through a canal and eleven rivers (Abbasi et al., 2017), which represent additional pathways of introduction through natural and human-assisted dispersal. In addition to extant NN fishes in the AWC, horizon species (i.e. not yet present but likely to enter the wetlands in the near future) need to be identified to inform NN species management plans (Copp et al., 2009; Roy et al., 2014).

Despite growing concerns about the potential detrimental effects of NN fishes in Iranian inland waters (Keivany, Manoochehr, Abbasi, & Abdoli, 2016), there has previously not been a scientific and reliable decision support tool available for use in Iran with which to assess the risk of invasiveness. However, the Aquatic Invasiveness Screening Kit v2.2 (AS-ISK: Copp et al., 2016a) offers users 32 language options, including Persian, for carrying out risk screenings. The aim of the present study was therefore to undertake a risk screening of extant and potential future NN freshwater fishes with regard to the AWC to identify which NN species are likely to pose a high risk of becoming invasive in the study area. The outcome of the present study is intended to inform and contribute to the management and conservation of this important Ramsar wetland.

2 | MATERIAL AND METHODS

2.1 | Risk screening

Hereafter, the risk assessment (RA) area comprises all four sectors (i.e. west, east, central and north) of the AWC, which is located in north Iran (37°52′N, 49°28′E: Figure 1) and covers ≈20,000 ha of southwest Caspian Sea lowlands. The AWC is a complex of large, shallow water bodies that support a vast variety of resident and migrant water birds as well as native fish (Abbasi et al., 2017). Together with the Siah Kesheem Protected Area and the Selke Wildlife Refuge, the AWC received Ramsar designation (Convention on Wetlands of International Importance) in 1975. Based on the Köppen-Geiger climate classification system (Peel, Finlayson, & McMahon, 2007), the AWC falls within climate type Csa, with at least one month of mean air temperature > 22°C and with the driest month of summer receiving < 40 mm of rainfall (Raziei, 2017).

Twenty-nine NN freshwater fish species were selected for risk screening to determine their potential invasiveness in the RA area according to the following criteria (Table 1): 1) "extant" species (n = 13), that is already present in the RA area; 2a) "proximity horizon" species (n = 14), that is present in close proximity to the RA area; and 2b) "potential horizon" species (n = 2), i.e. those not yet reported but likely to enter the RA area in the near future. The horizon species were selected using the CABI scanning tool (www.cabi.org/horizonscanningtool), with potential introduction vectors identified that are likely to be relevant to the RA area. Risk screening of these NN species was undertaken using the AS-ISK (Copp et al., 2016a), which is available for free download at www.cefas.co.uk/nns/tools/. The AS-ISK was created by combining the generic screening module of the European Non-native Species in Aquaculture Risk Analysis Scheme (Copp et al., 2016b) within the architecture of the Fish Invasiveness Screening Kit (FISK) v2 (Lawson, Hill, Scott, Vilizzi, & Copp, 2015). The AS-ISK decision-support tool was designed to comply with the "minimum standards" (Roy et al., 2018) for the assessment of NN species for

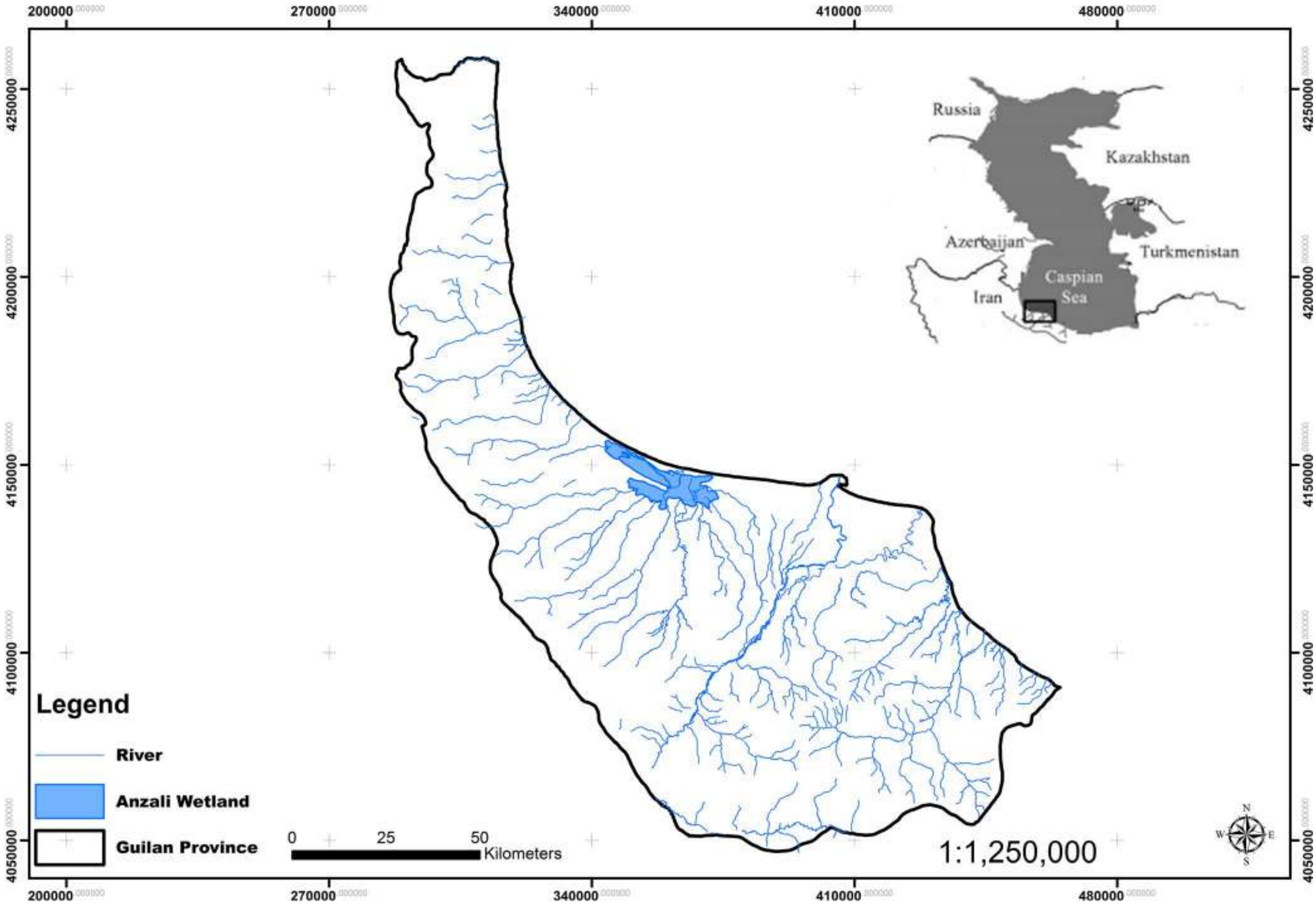

FIGURE 1 Map of the Anzali Wetland Complex, Iran

the EC Regulation No. 1143/2014 on the prevention and management of the introduction and spread of invasive alien species (Council of the European Communities, 2014).

The AS-ISK consists of 55 questions: the first 49 questions cover the biogeographical and biological aspects of the species under screening to achieve the Basic Risk Assessment (BRA) score; the other six Climate Change Assessment (CCA) 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. In the RA area under study, climate conditions at the end of the 21st Century are projected to be warmer than the current climate (Ebrahimi & Kardevani, 2014). To achieve a valid AS-ISK assessment, the assessor must provide a response, level of confidence in the response and a justification to each question (see below), resulting in a BRA score and a (composite) BRA + CCA score, respectively ranging from − 20 to 68 and from − 32 to 80. Confidence in responses to questions is ranked using a 1–4 scale (1 = low; 2 = medium; 3 = high; 4 = very high) as per the International Programme on Climate Change (Copp et al., 2016a; IPCC, 2005). The AS-ISK screenings were carried out separately by S.D. Moghaddas and H. Rahmani, who are knowledgeable in the environmental biology of the freshwater fishes of the RA area, with G.H. Copp and L. Vilizzi overseeing construction of the species list and quality control of the generated AS-ISK database of assessments, respectively.

2.2 | Data processing and statistical analysis

Basic AS-ISK scores <1 suggest that the species is unlikely (i.e. poses a low risk) to become invasive in the RA area, whereas scores >1 indicate a medium-to-high risk of the species becoming invasive in the RA area. The threshold values for the BRA and BRA + CCA that distinguish between medium- and high-risk levels are typically obtained through a procedure of RA area-specific "calibration," which is undertaken by Receiver Operating Characteristic (ROC) curve analysis (Bewick, Cheek, & Ball, 2004). For ROC curve analysis to be implemented, a minimum number of species needs to be screened (estimated to be 15–20: Vilizzi et al., 2019), and the species must be categorised a priori as "non-invasive" or "invasive" using independent literature sources. To this end, a priori categorisation for invasiveness was based on a four-step approach as follows: (a) similar to previous applications of the FISK to freshwater fishes (see Vilizzi et al., 2019) and AS-ISK (i.e. Bilge, Filiz, Yapici, Tarkan, & Vilizzi, 2019; Dodd, Vilizzi, Bean, Davison, & Copp, 2019; Glamuzina et al., 2017; Interesova, Vilizzi, & Copp, 2020; Li, Chen, Wang, & Copp, 2017; Tarkan, Sarı, İlhan, Kurtul, & Vilizzi, 2017a; Tarkan et al., 2017b; Uyan et al., 2020), a preliminary search was made of FishBase (Froese & Pauly, 2019) for any reference to the species' threat to humans, with the species categorised a priori as invasive if listed as "potential pest" and

TABLE 1 Non-native freshwater fish species assessed with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for the Anzali Wetland Complex

				Assessment component					Confidence					
				BRA		BRA + CCA			CL			CF		
Species name	Common name	Crit.	A priori	Score	Outcome	Score	Outcome	Delta	Total	BRA	CCA	Total	BRA	CCA
Abbottina rivularis[1]	Chinese false gudgeon	2b	Y	11.5	High	22.5	High	11.0	3.0	3.0	2.5	0.75	0.76	0.63
Acanthogobius flavimanus[2]	yellowfin goby	2b	Y	26.0	High	34.0	High	8.0	3.0	3.1	2.8	0.76	0.76	0.71
Anguilla anguilla	European eel	1	N	−1.0	Low	−6.0	Low	−5.0	3.3	3.3	3.0	0.82	0.83	0.75
Carassius auratus	goldfish	1	Y	39.0	High	51.0	High	12.0	3.3	3.3	3.3	0.83	0.83	0.81
Carassius carassius	crucian carp	2b	Y	31.8	High	43.8	High	12.0	3.2	3.2	3.0	0.79	0.80	0.75
Carassius gibelio	gibel carp	1	Y	38.5	High	50.5	High	12.0	3.6	3.7	3.0	0.90	0.91	0.75
Channa argus	northern snakehead	2b	Y	33.0	High	37.0	High	4.0	3.1	3.2	3.0	0.79	0.79	0.75
Chelon auratus	golden grey mullet	1	N	0.5	Low	−9.5	Low	−10.0	3.1	3.1	3.0	0.76	0.77	0.75
Chelon saliens	leaping mullet	1	N	2.5	Medium	4.5	Medium	2.0	3.4	3.4	3.0	0.85	0.86	0.75
Clarias batrachus	walking catfish	2b	Y	35.5	High	47.5	High	12.0	3.1	3.1	2.9	0.78	0.79	0.73
Ctenopharyngodon idella	grass carp	1	Y	15.0	High	27.0	High	12.0	3.3	3.3	3.0	0.82	0.82	0.75
Cyprinus carpio	common carp	1	Y	20.5	High	32.5	High	12.0	3.3	3.3	3.0	0.83	0.83	0.75
Gambusia affinis	western mosquitofish	2b	Y	28.0	High	39.0	High	11.0	3.1	3.2	2.8	0.78	0.79	0.71
Gambusia holbrooki	eastern mosquitofish	1	Y	27.5	High	39.5	High	12.0	3.4	3.5	3.0	0.86	0.87	0.75
Gasterosteus aculeatus	threespine stickleback	1	N	16.5	High	11.5	Medium	−5.0	3.3	3.3	3.0	0.82	0.83	0.75
Hemiculter leucisculus	sharpbelly	1	Y	29.0	High	41.0	High	12.0	3.4	3.4	3.3	0.85	0.85	0.81
Hypophthalmichthys molitrix	silver carp	1	Y	13.0	High	24.0	High	11.0	3.3	3.3	2.9	0.82	0.83	0.73
Hypophthalmichthys nobilis	bighead carp	1	Y	12.5	High	23.5	High	11.0	3.3	3.4	2.8	0.82	0.84	0.69
Ictalurus punctatus	channel catfish	2b	Y	30.5	High	42.5	High	12.0	3.0	3.1	2.8	0.76	0.76	0.71
Leuciscus idus[3]	ide (a.k.a. orfe)	2b	Y	21.5	High	27.5	High	6.0	3.1	3.1	3.0	0.76	0.77	0.75
Mylopharyngodon piceus[4]	black carp	2b	Y	15.0	High	21.0	High	6.0	3.1	3.1	3.0	0.78	0.78	0.75

(Continues)

TABLE 1 (Continued)

				Assessment component					Confidence					
				BRA		BRA + CCA			CL			CF		
Species name	Common name	Crit.	A priori	Score	Outcome	Score	Outcome	Delta	Total	BRA	CCA	Total	BRA	CCA
Neogobius fluviatilis[5]	monkey goby	2b	Y	10.5	High	12.5	High	2.0	3.0	3.0	2.8	0.75	0.76	0.71
Oncorhynchus mykiss	rainbow trout	2a	Y	20.0	High	12.0	High	−8.0	3.2	3.2	3.1	0.81	0.81	0.77
Oreochromis aureus[6]	blue tilapia	2b	Y	28.8	High	39.8	High	11.0	3.1	3.1	2.9	0.77	0.78	0.73
Perccottus glenii[7]	Chinese (Amur) sleeper	2b	Y	25.5	High	37.5	High	12.0	3.1	3.1	2.9	0.76	0.77	0.73
Poecilia latipinna[8]	sailfin molly	2b	Y	13.3	High	19.3	High	6.0	3.0	3.0	2.9	0.75	0.76	0.73
Pseudorasbora parva	topmouth gudgeon	1	Y	20.5	High	32.5	High	12.0	3.4	3.4	3.0	0.84	0.85	0.75
Rhinogobius similis	Amur goby	2a	Y	4.0	High	13.0	High	9.0	3.1	3.2	2.7	0.78	0.79	0.67
Rhodeus ocellatus[9]	rosy bitterling	2b	Y	10.5	High	18.5	High	8.0	3.1	3.1	3.0	0.77	0.78	0.75

Note: Authority: [1] (Basilewsky); [2] (Temminck & Schlegel); [3] (Linnaeus); [4] (Richardson); [5] (Pallas); [6] (Steindachner); [7] Dybowski; [8] (Lesueur); [9] (Kner).

For each species, the selection criterion (Crit.: (1) “extant,” that is, already present in the RA area; (2a) “proximity horizon,” that is, present in close proximity to the RA area; and (2b) “potential horizon,” that is, not yet reported but likely to enter the RA area in the near future), 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 are provided. Risk outcomes are based on a threshold of 3.25 for the BRA (Low: score within interval [−20, 1[; Medium: [1, 3.25[; High:] 3.25, 68]) and of 11.75 for the BRA + CCA (Low: [−32, 1[; Medium: [1, 11.75[; High:] 11.75, 80]) (note the reverse bracket notation indicating in all cases an open interval). The AS-ISK report for the screened species is provided in the Supplementary material. See also Figure 2.

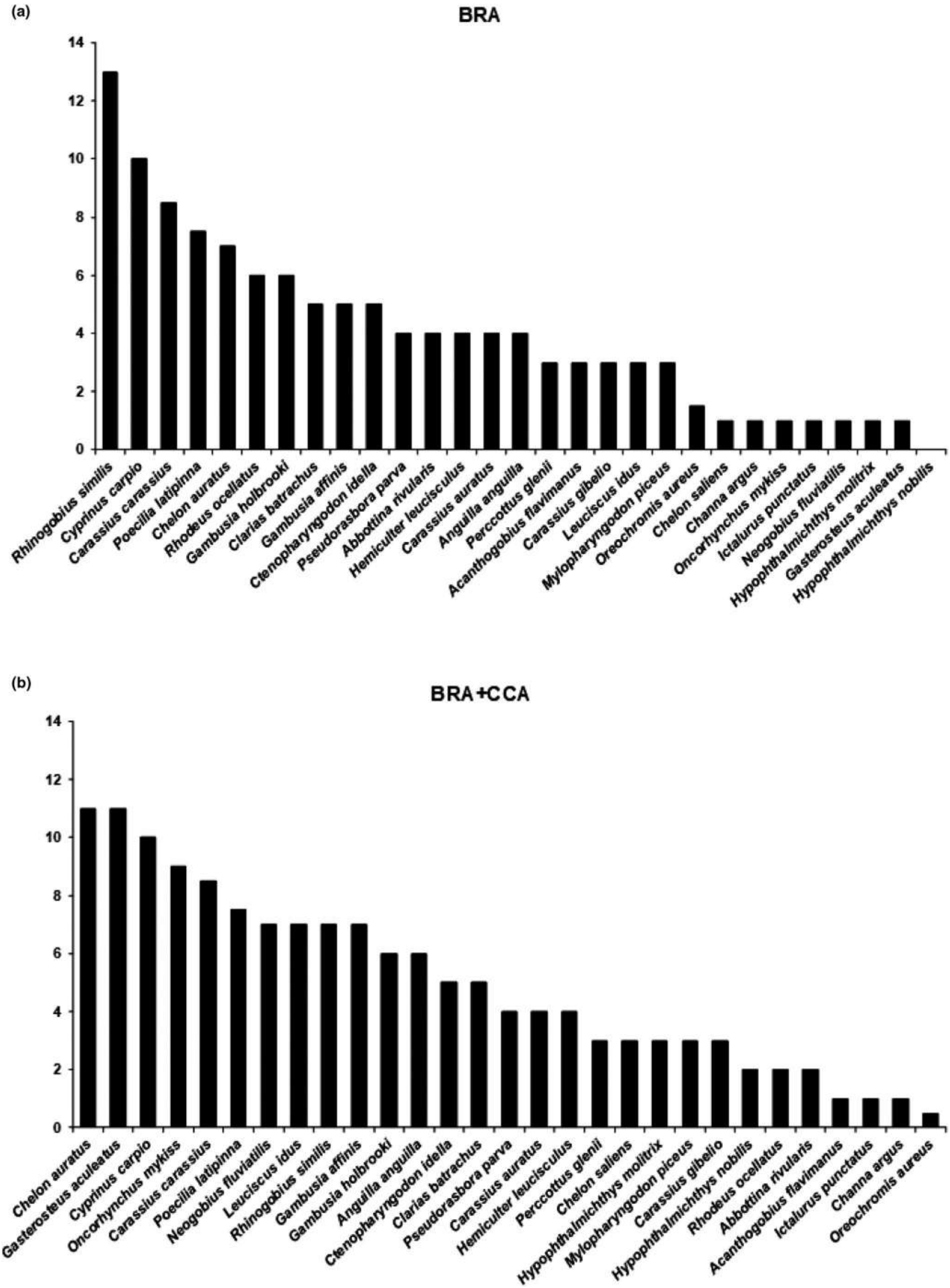

FIGURE 2 (a) Between-assessor differences in the BRA (Basic Risk Assessment) score for the species screened with the Aquatic Species Invasiveness Screening Kit for the Anzali Wetland Complex; (b) same for the BRA + CCA (Climate Change Assessment) score. See also Table 1

as non-invasive if listed as "harmless"; (b) in case the species was listed as either "not evaluated" or "absent" in the above database, then a search was made of the Global Invasive Species Database (GISD: www.iucngisd.org), with the species categorised a priori as invasive if listed therein; (c) in case the species was absent from the GISD, then an additional search was made of the continent-level lists for invasive species in "Africa," "Asia," "Europe," "North-America" and "South-Africa" (the lists for each of these can be obtained by adding the above-quoted continent name to the following URL: https://en.wikipedia.org/wiki/List_of_invasive_species_in_), whereby the species was categorised a priori as "invasive" if it appeared in the generated list; finally, (d) in case 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 taxon) 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 the species was categorised a priori as non-invasive if no evidence was found. Notably, in case 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.

Statistically, a ROC curve is a graph of sensitivity versus 1– specificity (or alternatively, sensitivity versus specificity) for each threshold value, where in the present context, sensitivity and specificity will be the proportion of a priori invasive and non-invasive fish species, respectively, that are correctly identified by 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, and 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). Following ROC 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-risk and medium-risk species. Initially, separate ROC curves for the BRA and the BRA + CCA were generated for the two assessors and differences between corresponding AUCs statistically tested (Mann–Whitney *U*-statistic, $\alpha = 0.05$) (online applet StAR: http://melolab.org/star/shome.php; Vergara, Norambuena, Ferrada, Slater, & Melo, 2008). ROC curve analysis was then carried out with package pROC (Robin et al., 2011) for R x64 v3.2.0 (R Development Core Team, 2019) using 2,000 bootstrap replicates for the confidence intervals of specificities, which were computed along the entire range of sensitivity points (i.e. 0 to 1, at 0.1 intervals).

Based on the confidence level (CL) allocated to each response (see *Risk screening*), a confidence factor (CF) is obtained as:

$CF = \sum(CL_{Qi})/(4 \times 55)$ ($i = 1, \ldots, 55$).

where CL_{Qi} 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 questions comprising the AS-ISK questionnaire. The CF ranges from a minimum of 0.25 (i.e. all 55 questions with confidence level equal to 1) to a maximum of 1 (i.e. all 55 questions with confidence level equal to 4). Finally, two additional CFs can be computed, namely the CF_{BRA} and the CF_{CCA}, respectively, based on the 49 Qs comprising the BRA and the six Qs comprising the CCA. Differences between mean CL_{BRA} and mean CF_{BRA} as well as between mean CL_{CCA} and mean 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 Euclidean distance measure, 9,999 unrestricted permutations of the raw data (Anderson, Gorley, & Clarke, 2008), and with statistical effects evaluated at $\alpha = 0.05$.

3 | RESULTS

There were no statistically significant differences between AUCs in either the BRA ($p = 0.238$) or the BRA + CCA ($p = 0.365$) for the two assessor-specific ROC curves. This justified computation of a global ROC curve based in both cases on the mean corresponding AS-ISK scores (Table 1). The ROC curve for the BRA resulted in an AUC of 0.9100 (0.7272–1.0000 95% CI) and that for the BRA + CCA in an AUC of 1. These AUCs were well above 0.5, indicating that the AS-ISK was able to discriminate reliably between non-invasive and invasive freshwater fish species for the RA area. Youden's *J* provided the thresholds of 3.25 for the BRA and 11.75 for the BRA + CCA, which were used for the calibration of risk outcomes. Accordingly, the BRA threshold allowed the distinction of medium-risk species with scores within the interval [1, 3.25[, from high-risk species with scores within]3.25, 68]; and the BRA + CCA threshold allowed distinction of medium-risk species with scores within [1.0, 11.75[from high-risk species with scores within]11.75, 80]. Whereas, species classified as low risk were those with BRA scores within [−20, 1[and BRA + CCA scores within]−32, 1] (note the reverse bracket notation indicating in all cases an open interval, as is statistically appropriate: www.mathwords.com/i/interval_notation.htm). The AS-ISK report for the screened species is provided in the Supplementary material document.

Of the 29 NN freshwater fish species assessed, based on the BRA threshold, 26 (89.7%) were classified as high risk, one (3.4%) as medium risk, and two (6.9%) as low risk (Table 1). All 25 species categorised a priori as invasive were classified as high risk (true positives), and of the four species categorised a priori as non-invasive, one species was a false positive (threespine stickleback *Gasterosteus aculeatus* Linnaeus). Finally, the lone species (*Chelon saliens*) categorised a priori as non-invasive was attributed a low-risk score (as a true negative).

Based on both the BRA + CCA threshold, 25 species (86.2%) were classified as high risk, two species (6.9%) as medium risk, and another two species (6.9%) as low risk. All 25 species categorised a priori as invasive were correctly classified as high risk (true positives), and amongst the four species categorised a priori as non-invasive no false positives were detected. Finally, two species categorised a priori as non-invasive were attributed medium-risk scores.

With regard to the BRA scores, the highest-scoring (invasive) species (score > 30, taken as an *ad hoc* "very high risk" threshold) were *Carassius auratus*, gibel carp *Carassius gibelio* (Bloch), Philippine catfish *Clarias batrachus* (Linnaeus), northern snakehead *Channa argus* (Cantor), crucian carp *Carassius carassius* (Linnaeus) and channel catfish *Ictalurus punctatus* (Rafinesque) (from higher to lower scores). Regarding the BRA + CCA scores, the highest-scoring (invasive) species (score > 40, same criterion as above) included *Hemiculter leucisculus* and all of the above species except *Channa argus*. *Chelon saliens* was classified as medium risk for both the BRA and the BRA + CCA, and *Gasterosteus aculeatus* only for the BRA + CCA. Further, for both the BRA and the BRA + CCA, European eel *Anguilla anguilla* (Linnaeus) and *Chelon auratus* were always classified as low risk, that is, score < 1 (Table 1). Differences in BRA scores between the two assessors ranged from 0 to 13, with a mean and median of 4.0, and 5% and 95% CIs of 1.0 and 9.4, respectively. Differences in BRA + CCA scores between assessors ranged from 0.5 to 11, with a mean and median of 4.0 and 5%, and 95% CIs of 1.0 and 10.6, respectively (Figure 2).

The CCA scores increased the BRA score for 25 (86.2%) of the screened species and decreased the score of the remaining four (13.8%) species. Notably, all highest-scoring species for both the BRA and the BRA + CCA also achieved the largest possible (positive) change in score of 12 (Table 1).

The mean CL (i.e. over all 55 Qs) was 3.19 ± 0.03 *SE*, the mean CL_{BRA} 3.22 ± 0.03 SE and the mean CL_{CCA} 2.95 ± 0.03 SE (hence, in all cases indicating high confidence), and the CL_{BRA} was significantly higher than the CL_{CCA} ($F^{\#}_{1,56} = 45.86$, $p < 0.001$). Similarly, the mean values for the CF = 0.798 ± 0.007 *SE* and $CF_{BRA} = 0.805 \pm 0.008$ *SE* were higher than the mean value for the $CF_{CCA} = 0.736 \pm 0.007$ *SE*, and the mean CF_{BRA} was significantly higher than the mean CF_{CCA} (same significance values as for the CL_{BRA} versus CL_{CCA} comparison due to the two indices being related). In all cases, the narrow standard errors indicated overall similarity in CLs and CFs across the species assessed.

4 | DISCUSSION

With regard to the AWC, the threshold AS-ISK score of 3.5 for discriminating between non-invasive and invasive species is considerably lower relative to the thresholds identified in two other AS-ISK screenings of freshwater fishes, namely for Turkey (Tarkan et al., 2017b) and the River Yarlung Zangbo, Tibetan Plateau, China (Li et al., 2017), which yielded BRA thresholds of 28 and 29, respectively. Whereas, not-so-dissimilar threshold scores were obtained for the River Neretva catchment (i.e. 10) in the Balkan region (Glamuzina et al., 2017) and for Lake Marmara (i.e. −3.5) in Turkey (Tarkan et al., 2017a). The relatively low AS-ISK threshold score obtained for the AWC may result from eight of the 15 NN species being present in the vicinity of, or at an early stage of invasion in, the RA area (Table 1). Of these eight species, rainbow trout *Onchorynchus mykiss* (Walbaum) and Amur goby *Rhinogobius similis* Gill have been reported to be present in rivers in close proximity to the RA area (Abbasi et al., 2017), and three species (*Anguilla anguilla*, *Chelon auratus* and *Chelon saliens*) are known to spawn outside of it, and another three species (*Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *Hypophthalmichthys nobilis*) are present in the RA area but unable to produce live gametes (Abdoli & Naderi, 2009).

Two members of the Genus *Carassius* (namely, *C. auratus* and *C. gibelio*) achieved the highest BRA and BRA + CCA scores amongst all NN fishes screened. Both species are widespread across the AWC (Abbasi et al., 2017), where they are considered invasive (Esmaeili et al., 2014). Overall, *C. auratus* and *C. gibelio* are capable of high tolerance to environmental changes and stresses, which underpins their global invasiveness (e.g. Copp, Wesley, & Vilizzi, 2005; Morgan & Beatty, 2007; Vetemaa, Eschbaum, Albert, & Saat, 2005; Yerli et al., 2014). Also, *C. gibelio* possesses the ability to reproduce gynogenetically, which makes this species a particularly successful invader (e.g. Lusková, Lusk, Halačka, & Vetešník, 2010; Tarkan, Cucherousset, Zięba, Godard, & Copp, 2010; Van der Veer & Nentwig, 2015; Yerli et al., 2014), and the species' population in the AWC is dominated by females (Abdoli & Naderi, 2009; Sayad Borani, Nezami, & Kiabi, 2001). *Carassius auratus* was unintentionally introduced to the AWC as a contaminant of Asian carp consignments in 1964 and has since increased in abundance (Holčík & Oláh, 1992). Some of the unique life-history traits of *C. auratus*, including easy adaptation to different kinds of food items, maturity at age 1+, high fecundity, polycyclic ripening of gonads and intermittent spawning, have contributed to its high abundance across the AWC (Holčík & Oláh, 1992) and elsewhere (e.g. Morgan & Beatty, 2007; Tarkan et al., 2010).

In the AWC, populations of both domesticated (introduced) and wild forms of common carp *Cyprinus carpio* Linnaeus are present, even though the latter have declined dramatically as a result of elevated fishing pressure (Abdoli & Naderi, 2009; Holčík & Oláh, 1992) combined with unfavourable spawning conditions (Holčík & Oláh, 1992). Domesticated *C. carpio* ranked as high risk in the present study and can genetically contaminate wild *C. carpio* populations (e.g. Chistiakov & Voronova, 2009; Khalili and Amirkolaie, 2010; Memiş & Kohlmann, 2006), but also detrimentally impact the aquatic ecosystem (Vilizzi, Tarkan, & Copp, 2015). However, there is no evidence of hybridisation between domesticated and wild forms of *C. carpio* in the AWC (Abbasi, 2018). Along with domesticated *C. carpio*, *Ctenopharyngodon idella*, *Hypophthalmichthys molitrix* and *Hypophthalmichthys nobilis* (also classified as high risk) have been introduced to the AWC as part of stocking programmes (Holčík & Oláh, 1992). Despite Asian carp species having failed to establish self-sustaining populations in the RA area, their introduction may still pose risks to the native fish species as they can alter the food webs of invaded systems (Sass et al., 2014) and host novel diseases (Pazooki & Masoumian, 2012; Shamsi, Jalali, & Aghazadeh Meshgi, 2009), some of which have been recorded for the first time in Iran (Sattari, Mokhayer, Khara, Nezami, & Shafii, 2007). Additionally, large-bodied non-native fishes, such as the Asian carps, can displace the native fishes from habitat simply by virtue of their larger size (e.g. Kessel, Dorenbosch, Boer, Leuven, & Van der Velde, 2011; Knapp, 1996).

Under climate change conditions (BRA + CCA score), the classification of *Gasterosteus aculeatus*, which was attributed a high-risk BRA score, dropped to the medium-risk category. *Gasterosteus aculeatus* was first reported for Iran in the south Caspian Sea basin (Coad & Abdoli, 1993), that is, the rivers Gorganrud and Tajan and the south-east corner of the Caspian Sea, including Gorgan Bay (Kiabi, Abdoli, & Naderi, 1999). Although this species' mode of introduction remains unknown (Coad, 2015), it is possible that *G. aculeatus* arrived as an unintentional contaminant of consignments of live fish imported for food consumption (Coad & Abdoli, 1993), with translocation as eggs or live fish by birds also a possibility (e.g. Boaz, 2009; Lovas-Kiss et al., 2020; Menezes, Ramos, Pereira, & Moreira da Silva, 1990).

Pseudorasbora parva, which attracted a high-risk BRA score and a very high-risk BRA + CCA score, was first reported for the south Caspian Sea basin in 1991. This highly invasive aquaculture pest (Abdoli & Naderi, 2009; Gozlan et al., 2010) has since become widely distributed and well established in the Caspian Sea (Coad & Abdoli, 1993), including in the AWC (Abbasi et al., 2017), due to its dispersal abilities and rapid establishment in water bodies (Gozlan et al., 2010).

Hemiculter leucisculus, which obtained a high-risk ranking for both the BRA and BRA + CCA scores, was first identified as a non-native fish in the AWC (and Iran) in 1990 (Holčík & Razavi, 1991), and its population has been found to be characterised by a good condition (i.e. plumpness) factor (Radkhah, Eagderi, & Asadi, 2016). Some of the life-history traits that contribute to this species being a successful invader include batch spawning, early maturation and an *r* reproductive strategy (Wang, Jakovlić, Huang, Wang, & Shen, 2016). *Hemiculter leucisculus* also consumes a wide variety of organisms and can easily switch to alternative food resources. Therefore, at high densities this species could represent a serious food competitor to native fish species in the AWC (Holčík & Razavi, 1991) and may pose risks as a new host for some parasites (Pazooki, Tajbakhsh, & Masoumian, 2011; Sattari et al., 2007).

Two species classified as high risk were *Gambusia holbrooki* and its congener western mosquitofish *Gambusia affinis* (Baird & Girard), which were treated as sub-species until they were designated as two separate species (Wooten, Scribner, & Smith, 1988). Similar to other parts of the world, *G. holbrooki* was deliberately introduced to the AWC (in 1922–1930) as a biological agent to control mosquito larvae responsible for malaria (Coad & Abdoli, 1993). This species has since dispersed and established widely throughout the AWC as well as the south Caspian Sea basin (Coad & Abdoli, 1993; Kiabi et al., 1999). High densities of *G. holbrooki* have the potential to alter plankton structure and dynamics (Blanco, Romo, & Villena, 2004; Macdonald et al., 2012; Margaritora, Ferrara, & Vagaggini, 2001), which could adversely affect native planktivorous fish in the AWC.

Chelon saliens (medium risk) and *C. auratus* (low risk) were translocated from the Black Sea to the Caspian Sea between 1930 and 1934 (Coad & Abdoli, 1993). In the AWC, *C. saliens* has a wider distribution than *C. auratus*, which is limited to the estuary (Abbasi et al., 2017). Although economically important to local fishermen (Abdoli & Naderi, 2009), neither of these species is known to reproduce in the RA area—their eggs develop in the open sea and juveniles feed along the southern Caspian Sea and the AWC (Coad, 2017). The other medium-risk species, *A. anguilla*, migrated into the Caspian Sea via the River Volga canal system, and it is now distributed to the west of the AWC with occasional catches reported by local fishermen (Abdoli & Naderi, 2009; Holčík & Razavi, 1991). Although not regarded as a food fish in Iran, *A. anguilla* catches have a restricted distribution in the Caspian Sea (Kiabi et al., 1999). This species appears to have declined recently (Abbasi et al., 2017), likely due to a decrease or cessation of stocking (e.g. Essl, Gassner, Schabetsberger, Jagsch, & Kaiser, 2016).

As a result of its long isolation and high level of endemism (Shiganova, 2010), the Caspian Sea region is at particular risk of species invasions (Leppäkoski et al., 2002; de Mora & Turner, 2004; Vodovsky, Patoka, & Kouba, 2017). These have adversely affected ecosystem function at all trophic levels (Shiganova, 2010; Zarbaliveva, Akhundov, Kasimov, Nadirov, & Hyseynova, 2016), and invasive species are amongst the leading threats to commercial and threatened native fish species, for example, sturgeons (Niksirat, Hatef, & Abdoli, 2010; Zarbaliyeva et al., 2016). In line with this issue, Article 12 of the Tehran Convention is dedicated to invasive species management (Prevention of Introduction, Control and Combating of Invasive Alien Species). In this regard, imported fish consignments and the aquarium trade are the two main vectors of intentional or unintentional introductions of NN fish species to the Caspian Sea (Leppäkoski et al., 2002) that can be relevant to wetlands of international importance in the region (e.g. Anzali and Miankaleh in Iran, Gizil-Aghaj in Azerbaijan, and the River Ural delta in Kazakhstan).

In the present study, the AS-ISK decision-support tool successfully identified NN freshwater fishes that pose a high risk of being or becoming invasive in the AWC. These results will provide decision-makers and managers with a basis upon which to formulate their management plans for this Ramsar site (and potentially other sites in Iran and the wider area represented by the Gulf region to the south of the country). The CCA component of AS-ISK also provides stakeholders with an evaluation of the likely impact that future climate conditions could have on the risk posed by the screened NN fishes to the RA area. Six of the 15 existing NN fishes in the RA area were ranked as posing a high risk of being invasive (i.e. *Carassius auratus*, *C. gibelio*, *Cyprinus carpio*, *Gambusia holbrooki*, *Hemiculter leucisculus* and *Pseudorasbora parva*) and therefore represent priority species for management and control. Given that prevention is more cost-effective than control and/or eradication (Lovell, Stone, & Fernandez, 2006), eight out of the 16 NN horizon species (including *Carassius carassius*, *Clarias batrachus* and *Ictalurus punctatus*) also pose a high risk of becoming invasive if introduced.

Gaps in knowledge of some NN fishes (e.g. *Chelon auratus* and *Rhinogobius similis*) were evident from the lower confidence rankings attributed to them (Table 1), thus highlighting areas for future research on NN fishes in the AWC so as to guide future policy and management decisions. Indeed, one of the main goals of the fourth Ramsar Convention Strategic Plan 2016–2024 (Goal 1, Paragraph 4) for Ramsar sites such as the AWC is the management of invasive NN species. This includes the identification of introduction pathways, priority species, and their control and management. This initial application of AS-ISK and horizon-screening exercise for the NN species in the Caspian region has demonstrated to be a potentially useful decision-support tool to assist managers and decision-makers in countries around the Caspian Sea as well as throughout the Middle East.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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