Research Article

Risk screening of non-native freshwater fishes in Yunnan Province, China

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Abstract

Invasive non-native freshwater fishes have long been recognized as a threat to biodiversity conservation and management. Non-native fishes with a high risk of becoming invasive can be identified using risk screening tools. Here, we used the Aquatic Species Invasiveness Screening Kit (AS-ISK) to identify the invasion risk of non-native fishes in Yunnan Province, China. AS-ISK scores were calibrated to distinguish between species with high and low-to-medium risks of invasiveness. Risk threshold was 17.25 for BRA (Basic Risk Assessment) and BRA+CCA (BRA+ Climate Change Assessment) in Yunnan Province. Based on BRA scores, 21 of the 37 screened species were classified as high risk, 13 as medium risk and 3 as low risk. Based on BRA+CCA scores, 22 of the 37 screened species were classified as high risk, 12 as medium risk and 3 as low risk. For both BRA and BRA+CCA, the highest-scoring species were *Hypostomus plecostomus*, *Oreochromis aureus*, *Oreochromis mossambicus* and *Oreochromis niloticus*. This study reliably assessed the risk of non-native fish invasion in Yunnan Province and identified priority non-native fishes for prevention and control, providing information for targeted monitoring and management decisions in the region.

Key words: AS-ISK, biological invasion, Risk threshold, invasion risk, Basic Risk Assessment, Climate Change Assessment, targeted monitoring and management

Introduction

Biological invasion is widely recognized as an important driver of global biodiversity loss (Pysek et al. 2010; Simberloff et al. 2013). The introduction of non-native fishes has resulted in a much greater biodiversity loss in freshwater than in terrestrial and marine ecosystems (Rocha et al. 2019; Tickner et al. 2020). China has the largest number of non-native freshwater fish in the world, with a total of 439 species introduced belonging to 67 families and 256 genera in 22 taxa, among which 53 species have established self-sustaining populations in the wild (Xiong et al. 2006). Recent studies have demonstrated that the introduction of non-native fishes and the extinction of native fishes have homogenized freshwater fish faunas across China. At the country scale, the similarity of fish species composition has increased by 7.0% (i.e. from 14.9% to 21.9%), with the highest degree of homogenization in the southwest ecological region (Liu et al. 2017). Non-native fishes may alter native fish assemblages through predation, competition, hybridization and disease transmission, causing ecological damage (Havel et al. 2015; Ribeiro and Leunda 2012). Once non-native fishes establish in aquatic environments, their post-invasion control is generally costly and extremely difficult (Williams and Grosholz 2008).

Risk screening, which can identify potentially invasive non-native species in a given risk assessment area, is a critical step in preventing or mitigating the impact of biological invasions and contributes to the development of policies and management procedures for specific risk assessment areas (Copp et al. 2016a). The Aquatic Species Invasiveness Screening Kit (AS-ISK) is a decision-support tool for the risk screening of non-native aquatic species, combining questions from the European Non-native Species in Aquaculture Risk Analysis Scheme's generic screening module (Roy et al. 2018). The AS-ISK has been extensively used in at least 120 risk assessment areas worldwide for screening some 819 non-native species from 15 groups of aquatic organisms and has proven to be an effective tool for identifying potentially invasive non-native species (Vilizzi et al. 2021). The outcome of AS-ISK consists of a Basic Risk Assessment (BRA) score and a BRA plus Climate Change Assessment (BRA+CCA) score. BRA scores can be used to calculate a calibrated (score-based) threshold that ranks species according to their risk level to identify high-risk species (Vilizzi et al. 2022). In general, AS-ISK can accurately distinguish the risk level of non-native species and provide the priority level of non-native species management for policy and decision-makers.

In southwest China, Yunnan Province has six major river systems and about thirty natural lakes. The unique combination of topographic complexity (altitude ranging from 76 to 6,740 m, with an average elevation of 2,000 m) and distinctive monsoon climate (annual mean temperature of ≈ 15 °C and annual mean precipitation of ≈ 1,000 mm) sustain an enormous biological diversity and a high degree of endemism (Wang and Dou 1998; Ye et al. 2015; Zhang 1999). However, Yunnan Province is one of the areas most seriously affected by the invasion of non-native fish in China. By 2013, 37 non-native fish species had been recorded in Yunnan Province. Most non-native fishes were introduced for fishery enhancement and a small part was brought in unintentionally during these introductions (Chen 2013). The extinction and homogenization of fish fauna caused by non-native fish invasion have become the biggest threat to biodiversity conservation in Yunnan Province. For example, the fish fauna of Lake Erhai, a typical plateau lake, was replaced by that of the middle and lower reaches of the Yangtze River, and indigenous fish species of Lake Erhai became endangered or extinct (Tang et al. 2013). However, compared with the severity of non-native fish invasion in Yunnan Province, systematic research is far from complete. Currently, there are no studies on the risk screening of non-native fish in Yunnan Province, which is a key step in the prevention and control of non-native fish.

With the promulgation of laws and regulations, such as the Biosafety Law of the People's Republic of China (2020) and the Regulations on Biodiversity Protection of Yunnan Province (2018), the introduction review of non-native fishes in Yunnan Province will be more scientifically sound and rigorous. In the future, non-native fish that can enter Yunnan Province will be reduced or even prevented to enter. This suggests that the control of non-native fishes in the area will focus more strongly on fishes that have arrived, especially those that have established stable populations in the wild.

The present study aimed to undertake a risk screening of non-native fish that have been recorded in Yunnan Province to identify which species are fish likely pose an elevated risk of invasion. The specific objectives were to: (a) conduct risk screenings of non-native fish using AS-ISK; (b) calibrate the resulting dataset to obtain the threshold for non-native fishes in Yunnan Province to distinguish high risk and low-to-medium risk species. The outcomes of this study are intended to identify priority non-native fishes for prevention and control, providing information for targeted monitoring and management decisions in the region.

Materials and methods

Study area

The risk screening area in this study, Yunnan Province, is in southwestern China, spanning latitudes 21°8′32″ to 29°15′8″N and longitudes 97°31′39″ to 106°11′47″E (Figure 1). It has a 394,100 km^2 area and 416.5 billion cubic meters of water resources. Yunnan Province is bordered by the Himalayan mountains to the north and the equatorial tropics to the south. It shares a western border with Myanmar and a southern border with Laos and Vietnam. A long and complex geomorphic process has created the unique and diverse geomorphic features of Yunnan Province. The western part of the province comprises mountains and rivers flowing from north to south, with peaks and valleys having relative altitudinal differences of up to 3,000 meters. The eastern part of the province is a limestone plateau with a karst landform formed by river erosion. Yunnan Province is dominated by the Yunnan-Guizhou Plateau in the north and tropical forests with low altitudes and a warm and humid climate in the south (Kang et al. 2013).

Yunnan Province has rich and diverse climate types, including all climate types from northern tropics to cold temperate zones. Yunnan Province has more than 600 rivers, including more than 180 major rivers. Most of them are the upper reaches of large rivers entering the sea and belong to the Jinsha River (upper reaches of the Yangtze River), the Nanpan River (source of the Pearl River), the Lancang River (Mekong River), the Nujiang River (Salween), the Irrawaddy River and the Yuanjiang River (Red River). In addition, the unique landform features of Yunnan Province form numerous natural lakes, among which there are nine lakes with an area of more than 30 km^2, including lakes Dianchi, Erhai, Fuxian, Lugu, Xingyun, Qilu, Chenghai, Yilong and Yangzonghai.

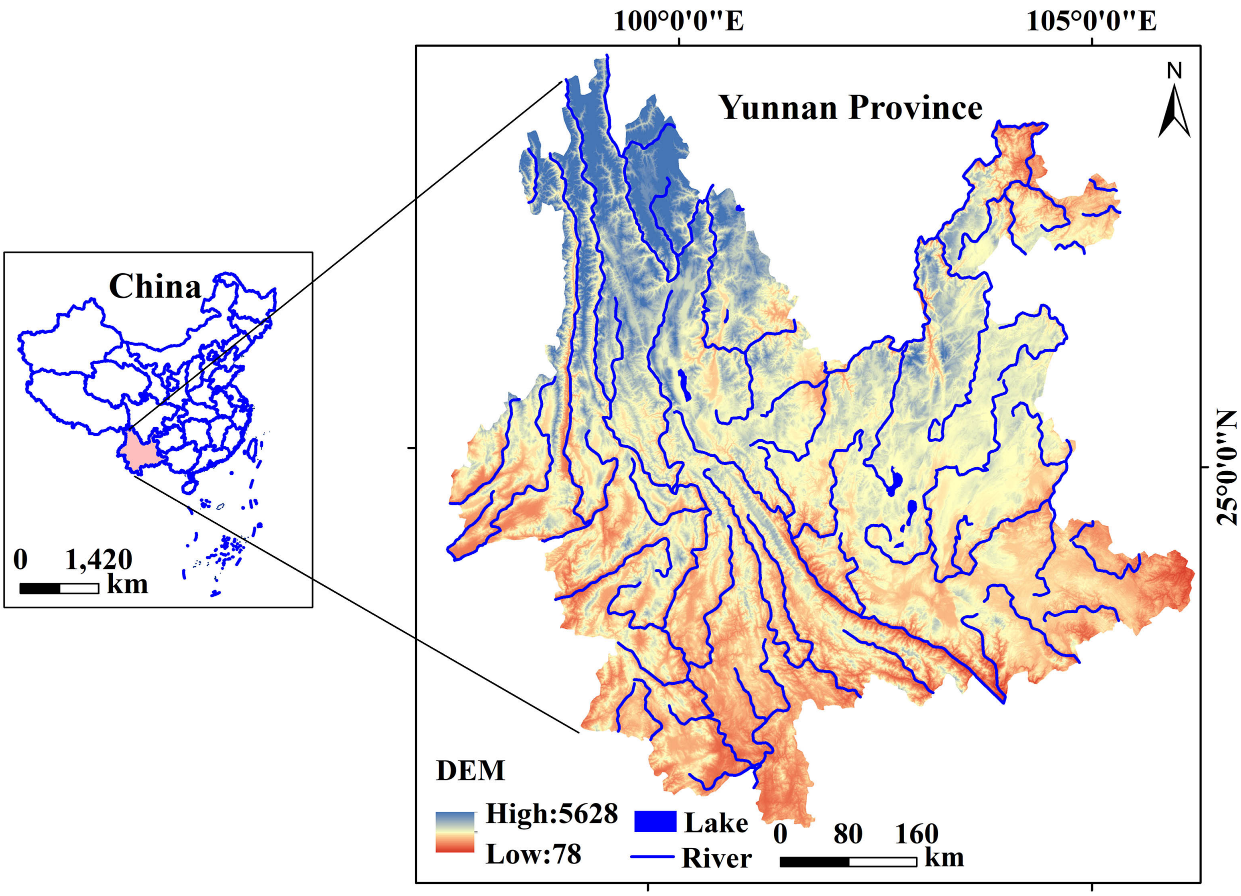

Figure 1. Map of the study (risk screening) area (Yunnan Province in southwest China)

Risk screening

We used AS-ISK v2.2 (www.cefas.co.uk/nns/tools/) to assess the potential invasion risk of non-native fishes in Yunnan Province (Copp et al. 2016b). Thirty-seven non-native freshwater fish species recorded in the study area were chosen for risk screening (Table 1). Thees fishes had been introduced to Yunnan Province according to the study by Chen (2013). AS-ISK requires assessors to answer 55 questions, including 49 basic questions and six questions about future climate change. The first 49 questions evaluate the biogeographical and biological properties of the species to estimate the risks of introduction, establishment, spread and impact, and result in the BRA score. The following six questions provide the BRA+CCA score and are used to determine the risk of species introduction, establishment, dispersal and impact under future climate change.

In addition to providing an answer to each question, the assessor must also give a corresponding justification and confidence level for each answer (see below). Screenings were carried out by the first author, who is knowledgeable in the biology and ecology of the fishes of the region. An AS-ISK score of < 1 indicates that the species is at little risk of invasion and has little invasiveness in the risk assessment area. When the AS-ISK score is > 1, the species is at a medium or high risk of invasion. Receiver Operating Characteristic (ROC) curve analysis is used to determine the threshold based on

Table 1. Non-native freshwater fish (with taxonomy) screened for their risk of invasiveness in Yunnan Province with the Aquatic Species Invasiveness Screening Kit (AS-ISK)

Order	Family	Taxon name	Common name	A priori categorisation	Native region
Anguilliformes	Anguillidae	*Anguilla anguilla*	European eel	Non-invasive	Europe
		Anguilla japonica	Japanese eel	Non-invasive	Asia
Acipenseriformes	Acipenseridae	*Acipenser baerii*	Siberian sturgeon	Invasive	Europe, Asia
Beloniformes	Hemiramphidae	*Hyporhamphus intermedius*	Asian pencil halfbeak	Non-invasive	China, Japan
Centrarchiformes	Centrarchidae	*Micropterus salmoides*	Largemouth bass	Invasive	North America
Characiformes	Serrasalmidae	*Piaractus brachypomus*	Pirapitinga	Invasive	Brazil, Colombia
	Prochilo-dontidae	*Prochilodus lineatus*	Streaked prochilod	Non-invasive	Brazil, Chile
	Cyprinidae	*Acheilognathus chankaensis*	Xingkai bitterling	Non-invasive	China
		Acheilognathus macropterus	Largefin bitterling	Non-invasive	China
		Carassius cuvieri	Japanese white crucian carp	Invasive	Japan
		Ctenopharyngodon idella	Grass carp	Invasive	China
		Cultrichthys erythropterus	Redfin culter	Non-invasive	China
		Hypophthalmichthys molitrix	Silver carp	Invasive	China
		Hypophthalmichthys nobilis	Bighead carp	Invasive	China
		Labeo rohita	Roho labeo	Non-invasive	India, Myanmar
		Megalobrama amblycephala	Wuchang bream	Non-invasive	China
		Mylopharyngodon piceus	Black carp	Invasive	China
		Parabramis pekinensis	White Amur bream	Non-invasive	China
	Cobitidae	*Paramisgurnus dabryanus*	Taiwaninmutakala	Non-invasive	China
	Cyprinidae	*Sarcocheilichthys nigripinnis*	Rainbow gudgeon	Non-invasive	China
		Tinca tinca	Tench	Invasive	Europe
		Toxabramis swinhonis	Thin sharpbelly	Non-invasive	China
Cyprinodontiformes	Poeciliidae	*Gambusia affinis*	Western mosquitofish	Invasive	America
Osmeriformes	Osmeridae	*Hypomesus olidus*	Pond smelt	Non-invasive	China, Japan
	Salangidae	*Neosalanx taihuensis*	Taihu icefish	Invasive	China
		Protosalanx hyalocranius	Clearhead icefish	Non-invasive	China
Perciformes	Cichlidae	*Oreochromis aureus*	Blue tilapia	Invasive	Africa
		Oreochromis mossambicus	Mozambique tilapia	Invasive	Africa
		Oreochromis niloticus	Nile tilapia	Invasive	Africa
	Gobiidae	*Rhinogobius cliffordpopei*	Goby	Invasive	China
	Sinipercidae	*Siniperca chuatsi*	Chinese perch	Non-invasive	China
Salmoniformes	Salmonidae	*Oncorhynchus mykiss*	Rainbow trout	Invasive	North America
		Salmo trutta	Brown trout	Invasive	Europe
Siluriformes	Ictaluridae	*Ameiurus nebulosus*	Brown bullhead	Invasive	Canada, America
	Clariidae	*Clarias gariepinus*	North African catfish	Invasive	Africa
	Loricariidae	*Hypostomus plecostomus*	Suckermouth catfish	Invasive	South America
	Ictaluridae	*Ictalurus punctatus*	Channel catfish	Invasive	North American

BRA scores to distinguish between medium and high risk (Vilizzi et al. 2022). The Area Under the Curve (AUC), which normally varies from 0.5 to 1, is a measure of the calibration analysis' accuracy; the closer to 1, the better the capacity to distinguish between invasive and non-invasive species.

A confidence factor (*CF*) was calculated using the confidence level (*CL*) (1 = low; 2 = medium; 3 = high; 4 = extremely high) assigned to each response (Vilizzi et al. 2022). Two more *CF*s, the CF_{BRA} and the CF_{CCA}, were calculated based on the 49 questions in the BRA and the six questions in the CCA, respectively.

Before ROC curve analysis, species must be categorised a priori according to existing records to distinguish their invasiveness (i.e., non-invasive or invasive). The a priori categorisation of the species was implemented based on a four-step approach (for full details of the methodology see Vilizzi et al. (2022) (Table 1). Implementation of the ROC curve analysis followed the protocol described in Vilizzi et al. (2022). Following ROC curve analysis, Youden's *J* statistic was used to identify the optimal AS-ISK threshold value that maximizes true positives while minimizing false positives. The default threshold of 1 was selected to distinguish between low-risk and medium-risk species.

The confidence intervals of specificities were estimated using 2000 bootstrap replicates for the confidence intervals of specificities, which were computed for the whole range of sensitivity points (i.e., 0 to 1, at 0.1 intervals), using the package "pROC" (Robin et al. 2011) for Rx64 v3.2.0. Permutational ANOVA based on a one-factor design was used to evaluate differences between mean CL_{BRA} and mean CF_{BRA}, as well as mean CL_{CCA} and mean CF_{CCA} (i.e., components with two levels: BRA and CCA). The analysis was carried out using the Rx64 v3.2.0 "vegan" package, with data normalization and a Euclidean distance measure, 9,999 unrestricted permutations of the raw data and statistical effects evaluated at 0.05.

Results

According to ROC curve results (Figure 2), the AUC was 0.9643 (0.9032–1.0000, 95% CI). The area under the ROC curve was consistently greater than 0.5, indicating that AS-ISK could accurately distinguish between medium and high risk non-native fishes in the risk assessment area. For the calibration of risk outcomes, Youden's *J* provided the threshold of 17.25. As a result, the threshold distinguished medium-risk species with scores in the range [1, 17.25[from high-risk species with scores in the range]17.25, 70] for the BRA and in the range]17.25, 82] for the BRA + CCA. Species identified as low risk had scores within [−20, 1[. The Supplementary materials (Table S1, Table S2 etc) include the AS-ISK report for the screened species.

Based on the BRA scores, 21 (56.8 %) of the 37 non-native fish species screened were identified as high risk, 13 (35.1%) as medium risk and 3 (8.1%) as low risk. There were 20 true positives and no false negatives among the 21 species categorized as invasive a priori. Twelve of the 13 medium-risk species were classified as non-invasive and one as invasive. One of the 16 species classified as non-invasive a priori were false positives, whereas two were true negatives. Twenty-two species (59.5%) were identified as high risk, 12 (32.4%) as medium risk and 3 (8.1%) as low risk based on the BRA+CCA scores. There were 19 true positives and no false negatives among the 21 species categorized as invasive a priori. Ten medium-risk species were all identified as non-invasive. Three of the 16 species categorized as non-invasive a priori were false positives, whereas 3 were true negatives (Table 2).

For the BRA, the highest risk species (based on an ad hoc "very high risk" threshold > 40) were *Hypostomus plecostomus*, *Oreochromis aureus, Oreochromis niloticus* and *Oreochromis mossambicus* (from high to low scores).

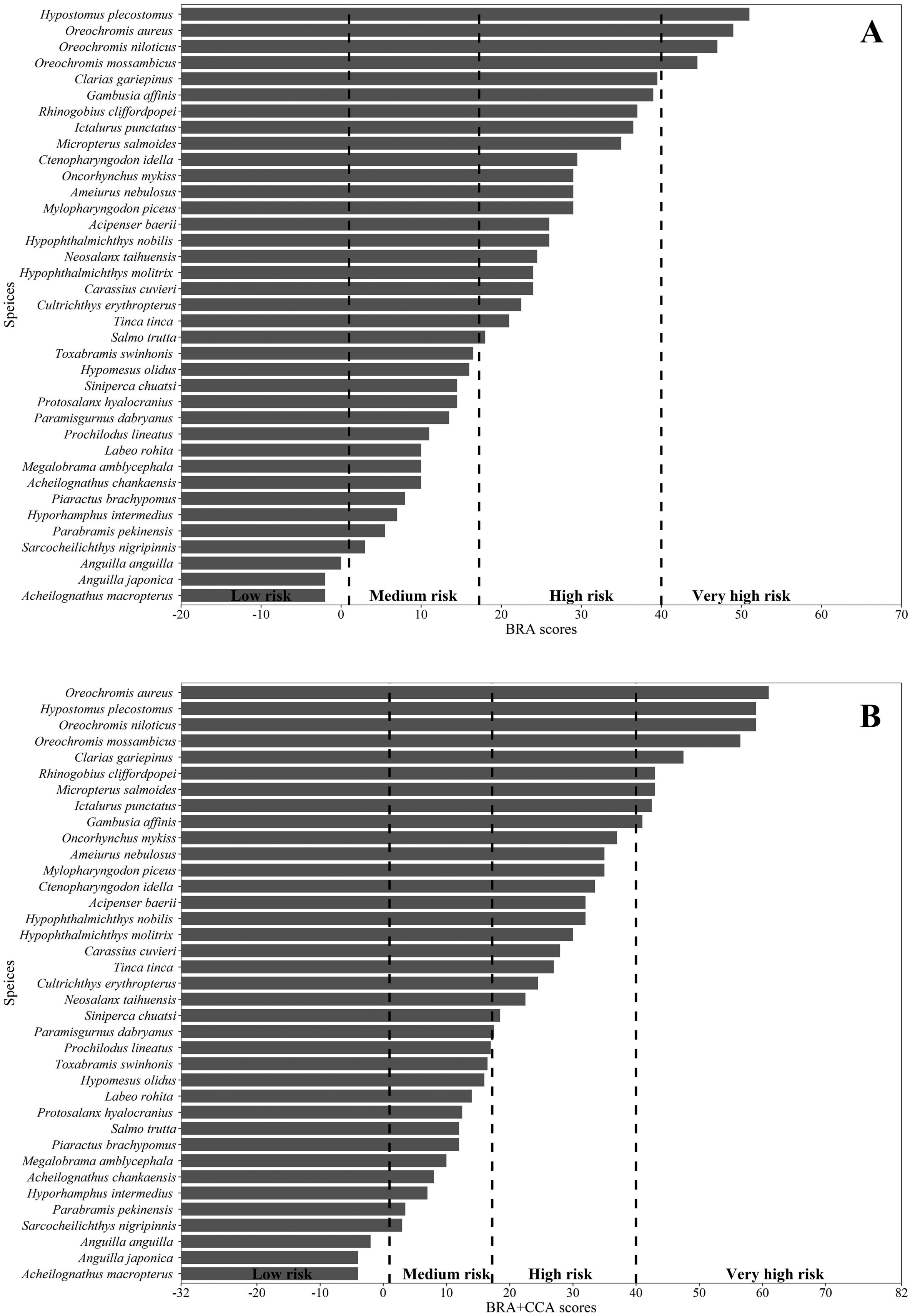

Figure 2. Risk screening scores for the non-native fish species screened with the AS-ISK: (A) basic risk assessment (BRA) for Yunnan Province; (B) BRA plus climate-change assessment (BRA+CCA) for Yunnan Province; dashed lines indicate thresholds for different intrusion risk levels (see thresholds in Table 2)

Table 2. Non-native freshwater fish species assessed with the Aquatic Species Invasiveness Screening Kit (AS-ISK) for Yunnan Province

Species name	A priori categorisation	Screening component: BRA Score	BRA Outcome	BRA+CCA Score	BRA+CCA Outcome	Delta	Confidence: CL Total	CL BRA	CL CCA	CF Total	CF BRA	CF CCA
Acheilognathus chankaensis	N	10	Medium	8	Medium	−2	3.4	3.4	3.5	0.85	0.85	0.88
Acheilognathus macropterus	N	−2	Low	−4	Low	−2	3.3	3.3	3.2	0.81	0.82	0.79
Acipenser baerii	Y	26	High	32	High	6	3.0	3.0	2.8	0.74	0.74	0.71
Ameiurus nebulosus	Y	29	High	35	High	6	3.0	3.0	2.8	0.75	0.76	0.71
Anguilla anguilla	N	0	Low	−2	Low	−2	3.0	3.0	3.0	0.77	0.77	0.75
Anguilla japonica	N	−2	Low	−4	Low	−2	3.1	3.2	3.0	0.79	0.80	0.75
Carassius cuvieri	Y	24	High	28	High	4	3.1	3.1	2.8	0.78	0.79	0.71
Clarias gariepinus	Y	39.5	High	47.5	High	8	3.1	3.1	2.8	0.77	0.78	0.71
Ctenopharyngodon idella	Y	29.5	High	33.5	High	4	3.3	3.3	3.2	0.83	0.84	0.79
Cultrichthys erythropterus	N	22.5	High	24.5	High	2	3.2	3.3	3.0	0.81	0.82	0.75
Gambusia affinis	Y	39	High	41	High	2	3.1	3.1	3.0	0.78	0.78	0.75
Hypomesus olidus	N	16	Medium	16	Medium	0	3.2	3.2	3.0	0.79	0.80	0.75
Hypophthalmichthys molitrix	Y	24	High	30	High	6	3.3	3.3	3.0	0.83	0.84	0.75
Hypophthalmichthys nobilis	Y	26	High	32	High	6	3.3	3.4	3.0	0.84	0.85	0.75
Hyporhamphus intermedius	N	7	Medium	7	Medium	0	3.2	3.2	3.0	0.80	0.80	0.75
Hypostomus plecostomus	Y	51	High	59	High	8	3.0	3.0	3.0	0.75	0.74	0.75
Ictalurus punctatus	Y	36.5	High	42.5	High	6	3.0	3.0	2.7	0.75	0.76	0.67
Labeo rohita	N	10	Medium	14	Medium	4	3.1	3.2	3.0	0.79	0.79	0.75
Megalobrama amblycephala	N	10	Medium	10	Medium	0	3.4	3.4	3.3	0.84	0.84	0.83
Micropterus salmoides	Y	35	High	43	High	8	3.1	3.2	2.5	0.79	0.81	0.63
Mylopharyngodon piceus	Y	29	High	35	High	6	3.3	3.3	3.0	0.82	0.83	0.75
Neosalanx taihuensis	N	24.5	High	22.5	High	−2	3.4	3.4	3.0	0.85	0.86	0.75
Oncorhynchus mykiss	Y	29	High	37	High	8	3.0	3.0	2.8	0.75	0.75	0.71
Oreochromis aureus	Y	49	High	61	High	12	3.1	3.1	2.7	0.77	0.78	0.67
Oreochromis mossambicus	Y	44.5	High	56.5	High	12	2.9	3.0	2.8	0.75	0.75	0.71
Oreochromis niloticus	Y	47	High	59	High	12	2.9	3.0	2.5	0.74	0.75	0.63
Parabramis pekinensis	N	5.5	Medium	3.5	Medium	−2	3.3	3.3	3.0	0.81	0.82	0.75
Paramisgurnus dabryanus	N	13.5	Medium	17.5	High	4	3.2	3.2	3.0	0.80	0.80	0.75
Piaractus brachypomus	Y	8	Medium	12	Medium	4	3.1	3.1	3.0	0.77	0.77	0.75
Prochilodus lineatus	N	11	Medium	17	Medium	6	3.1	3.1	3.0	0.76	0.77	0.75
Protosalanx hyalocranius	N	14.5	Medium	12.5	Medium	−2	3.1	3.1	2.8	0.76	0.77	0.71
Rhinogobius cliffordpopei	Y	37	High	43	High	6	3.1	3.1	2.8	0.76	0.77	0.71
Salmo trutta	Y	18	High	12	Medium	−6	2.9	2.9	3.0	0.73	0.72	0.75
Sarcocheilichthys nigripinnis	N	3	Medium	3	Medium	0	3.5	3.5	3.2	0.86	0.87	0.79
Siniperca chuatsi	N	14.5	Medium	18.5	High	4	3.0	3.0	2.8	0.74	0.74	0.71
Tinca tinca	Y	21	High	27	High	6	3.1	3.1	3.0	0.77	0.77	0.75
Toxabramis swinhonis	N	16.5	Medium	16.5	Medium	0	3.3	3.4	3.2	0.84	0.84	0.79

A priori categorisation (N: non-invasive; Y: invasive); Screening component (BRA: Basic Risk Assessment, BRA+CCA: BRA+ Climate Change Assessment, Delta: BRA+CCA score minus BRA score); Confidence (CL: confidence level, CF: confidence factor); Outcome (Low: score < 1, Medium: 1 ≤ score < 17.25, High: score ≥ 17.25)

As for BRA+CCA scores, the highest-scoring (invasive) species (threshold > 40) were *Oreochromis aureus, Hypostomus plecostomus*, *Oreochromis niloticus*, *Oreochromis mossambicus*, *Clarias gariepinus*, *Rhinogobius cliffordpopei*, *Micropterus salmoides*, *Ictalurus punctatus* and *Gambusia affinis* (from high to low scores). Low-risk species according to BRA scores (*Anguilla*

anguilla, *Anguilla japonica* and *Acheilognathus macropterus*) remained low risk according to BRA+CCA scores. The CCA resulted in an increase relative to the BRA score for 24 species (64.9%), a decrease for eight species (21.6%) and no change for the remaining five species (13.5%). Notably, three tilapia species (*Oreochromis niloticus, Oreochromis aureus* and *Oreochromis mossambicus*) showed the largest possible increase in score, i.e., 12 points.

Mean CL values (CL ± SE) were $CL = 3.1 \pm 0.15$ (i.e., overall 55 Qs), $CL_{BRA} = 3.2 \pm 0.16$ and $CL_{CCA} = 3.0 \pm 0.20$; hence, medium-to-high confidence in all cases. The CL_{BRA} was significantly higher than the CL_{CCA} ($F = 24.55$, $P < 0.001$). Similarly, mean CF (0.788 ± 0.037) and CF_{BRA} values (0.793 ± 0.039) were higher than the mean CF_{CCA} (0.739 ± 0.048). The mean CF_{BRA} was significantly higher than the mean CF_{CCA} (same significance values as for the CL_{BRA} *vs.* CL_{CCA} comparison due to the two indices being related). The low standard errors indicated overall similarity in CLs and CFs across the species screened in all cases.

Discussion

AS-ISK is considered to reliably assess the invasiveness of non-native fishes and has been widely used in many regions and countries worldwide (Vilizzi et al. 2021). In this study, twenty of the 21 fish species categorized a priori as invasive were classified as high risk (true positive) and none as low risk (false positive) based on ROC analysis. Furthermore, ROC analysis revealed one false-negative fish species (*Cultrichthys erythropterus*) categorized a priori as non-invasive. *Cultrichthys erythropterus* has established stable populations and is abundant in Yunnan Province (Ye et al. 2015). These results indicate that the AS-ISK decision support tool could be effectively applied to screen the risk of non-native fish in Yunnan Province.

The risk threshold based on BRA established for Yunnan Province (17.25) was lower than those of three other areas in China where risk assessments have been performed (the middle reach of the Yarlung Zangbo River, Haihe River Basin and the lower Pearl River basin) (Li et al. 2017, 2021; Wei et al. 2021). Although our threshold is higher than the thresholds established by Wei et al. (2022) (a case study of non-native Loricariidae species in China) and Li et al. (2023) (invasion risk assessment of exotic sturgeon and paddlefish in the Yangtze River Basin), the strong adaptability of these screened species is the main reason for their lower risk thresholds. Our lower risk threshold suggest that Yunnan Province is more vulnerable to non-native fish, mainly due to its diverse climate types and highly specialized fish species. With its diverse and geographical features, the province maintains a high level of biodiversity (Kang et al. 2013), but also provides a suitable environment for many non-native species. In addition to suitable habitat conditions, the success of invasions is also related to the intruder's biological and ecological characteristics and the community's vulnerability

(Shuai et al. 2018; Tilman 2004). Furthermore, highly specialized indigenous fish communities exhibit poor stability when facing external disturbances. Unfortunately, due to overfishing, habitat destruction and water pollution in recent decades, almost two-thirds of the indigenous species of the province have been listed as severely endangered or extinct (Chen et al. 1998; Ye et al. 2015). In contrast, most of the non-native fishes in Yunnan Province have been introduced to other parts of the world as economic fish, with strong environmental adaptability, phenotypic plasticity, fertility and competitiveness (Li et al. 2016). These factors are important reasons for the low risk threshold in Yunnan Province.

The species identified as the highest invasion risk under current and future climate conditions were tilapias (*Oreochromis aureus*, *Oreochromis mossambicus* and *Oreochromis niloticus*) and *Hypostomus plecostomus*. Tilapias are considered one of the world's most widely distributed group of invasive fish and classified as a potential pest in most tropical and subtropical countries (Canonico et al. 2005; Costa-Pierce 2003; Vitule et al. 2009; Zengeya et al. 2013). Screening results in Europe (Almeida et al. 2013; Ferincz et al. 2016; Perdikaris et al. 2016; Piria et al. 2016), Asia (Moghaddas et al. 2020; Ruykys et al. 2021; Tarkan et al. 2017), North America (Lawson et al. 2015) and the Yangtze River Basin in China (Li et al. 2023) were generally consistent with this study, indicating a moderately high- to- high risk of invasion of tilapias. In China, tilapias have escaped into the wild and formed stable populations, extending to most river systems in southern China (Blackburn et al. 2011; Shuai et al. 2017). Tilapias also escaped into the wild in Yunnan Province and have become the dominant species (Wang et al. 2011). High physiological tolerance, flexible habitat requirements, reproductive strategies, rapid growth and omnivorous habits enable tilapia to adapt quickly to the environment and establish sustainable populations (Canonico et al. 2005; Martin et al. 2010). So far, there have been few reports on the ecological impact of tilapia invasion in Yunnan Province. According to studies in Guangdong Province, declines in fish species diversity and functional diversity, as well as in fishery yield, were related to the invasion of tilapias (Gu et al. 2015; Shuai et al. 2019). *Hypostomus plecostomus* are not as invasive as tilapias globally, but they may be more invasive than tilapias in China. This species was not only the fish with the highest risk score in Yunnan Province but also in Vietnam (Ruykys et al. 2021). *Hypostomus plecostomus* were introduced to Yunnan Province as ornamental fish, escaped to the wild and formed stable populations. They have little economic value to the fishers and are thrown back into the river (Wei et al. 2019). This greatly reduces the fishing pressure and facilitates invasion. Moreover, *Hypostomus plecostomus* could adjust their life-history traits to increase fitness in stressful environments and have even been recorded in northern China (Wei et al. 2017). With global warming, the distribution range of *Hypostomus plecostomus* in China could expand further north (Britton et al. 2010).

Other high-scoring fishes can be divided into four categories according to the purpose of introduction: (1) introduced into lakes to enhance fishery production (*Ctenopharyngodon idella*, *Cultrichthys erythropterus*, *Hypophthalmichthys nobilis*, *Mylopharyngodon piceus* and *Neosalanx taihuensis*); (2) introduced into ponds and escaped to the wild (*Acipenser baerii*, *Ameiurus nebulosus*, *Carassius cuvieri*, *Clarias gariepinus*, *Ictalurus punctatus*, *Micropterus salmoides*, *Oncorhynchus mykiss*, *Salmo trutta* and *Tinca tinca*); (3) introduced for biological control (*Gambusia affinis*); and (4) introduced accidentally (*Rhinogobius cliffordpopei*).

Introduced into Yunnan lakes to enhance fishery production were mainly commercial fishes from other regions of China. *Neosalanx taihuensis* have undergone three large-scale artificial transplants in history and successfully established populations in Yunnan plateau lakes, producing great economic benefits (Kang et al. 2015). However, the introduction of *Neosalanx taihuensis* has induced abundance declines and alterations in zooplankton species composition (Liu et al. 2009). The competitive advantages of *Neosalanx taihuensis* with native fish in food resources and living space are believed to be the cause of the decline (or even extinction) of many endemic fish (Qin et al. 2007). For example, in Fuxian Lake, pressure from *Neosalanx taihuensis* caused a sharp decline in the annual yield of rare *Anabarilius grahami* from 400 to 500 tons in the 1960s to less than 1 ton in 2002 (Xiong et al. 2006).

In contrast, Asian carps (i.e., *Hypophthalmictuthys molitrix*, *Ctenopharyngodon idellus*, *Hypophthalmichthys nobilis* and *Mylopharyngodon piceus*) had a much lower invasion risk than *Neosalanx taihuensis* in Yunnan plateau lakes because the hydrologic conditions of the lakes do not allow them to spawn (Cuddington et al. 2014). However, Asian carps have escaped into Yunnan rivers and established populations, leading to high risk levels of Asian carps. In the United States, Asian carps have spread through large rivers in eastern and central North America. They are high-risk invaders to the Great Lakes because of their r-selected characteristics (e.g., rapid growth, fast dispersal capabilities, high reproductive potential, absence of natural predators and broad environmental tolerance) (Chapman et al. 2021; Phelps et al. 2017). Compared with BRA and BRA+CCA scores of Asian carps in the Anzali Wetland Complex of Iran and Turkey, the scores of Asian carps in this study were between those of the two regions (Moghaddas et al. 2020; Tarkan et al. 2017). The risk of Asian carp invasion depends largely on whether they can enter rivers and complete their life history.

The high-risk fishes that escaped after being introduced into ponds came mainly from other countries. In Yunnan Province, these fishes have not yet become dominant species in the wild, but their invasive abilities and ecological damage in other regions of the world suggest that they deserve our attention. For example, since 2004, *Micropterus salmoides* has been included in the list of “100 of the world’s worst invasive alien species” by

IUCN because of its role in fish fauna degradation and the extinction of native fish (Khosa et al. 2019; Lowe 2000). Also listed by IUCN as the worst invasive alien species was *Oncorhynchus mykiss*, which has been introduced worldwide (Crawford and Muir 2008). Many studies have found that non-native *Oncorhynchus mykiss* have significant detrimental effects on native fish species across continents (i.e., hybridization, disease transmission, predation and competition) (Nomoto et al. 2010; Shelton et al. 2017; Stankovic et al. 2015).

Moreover, these high-risk fishes are generally omnivorous and have high reproductive and environmental adaptability, which may allow them to establish populations in the natural environment. For example, *Clarias gariepinus* could inhabit nearly all habitats ranging from deep and shallow lakes to rivers and swamps (Vitule et al. 2006). In its natural range, its diet includes fish, invertebrates, plants, plankton, reptiles and amphibians (Kadye and Booth 2012). In addition, it exhibits seasonal reproduction with high fecundity and growth rate and has pseudo-lungs that allow it to adapt easily to new environments and even escape from aquaculture ponds by land (Winemiller and Kelso-Winemiller 1996). However, once these high-risk fishes escape into the environment and establish populations, actions such as eradication, control and containment are generally difficult and unlikely to be successful (Williams and Grosholz 2008). Therefore, it is imperative to accurately identify the risk levels of alien fish invasions and develop effective measures to block their spread.

Gambusia affinis is a small fish native to North America introduced as a biological mosquito control agent into the fresh and saltwater systems of all continents except Antarctica (Pyke 2008). As in other parts of the world, from the 1950s on, the *Gambusia affinis* was introduced to almost all of China (the Pearl River basin, Yangtze River basin and Yellow River basin) for mosquito control (Xiong et al. 2019). This species has since dispersed and established widely throughout the lakes and rivers of Yunnan Province and the southern waters of China (Liu et al. 2017). The invasion of *Gambusia affinis* has reduced the abundance and richness of invertebrates (Shulse et al. 2013), small native fishes (Ayala et al. 2007) and even amphibians (Segev et al. 2009). Moreover, higher temperatures increase its feeding rates and aggressiveness (Pyke 2005; Rincon et al. 2002) and promote its reproduction (Pyke 2005), which aggravates the destruction of biodiversity by *Gambusia affinis* in Yunnan Province.

Rhinogobius cliffordpopei, originally native to central and eastern China, was accidentally introduced into most lakes of the Yunnan Province in the 1950–1960s and quickly established populations (Xie et al. 2001). Similar to other parts of the world, the invasion of goby is considered one of the major causes of the decline or extirpation of some native fishes (Firth et al. 2021). For example, in Europe, round goby (*Neogobius melanostomus*) has led to the decline of three-spined stickleback (*Gasterosteus aculeatus*) in the Gulf of Gdansk and the protected river bullhead (*Cottus perifretum*) in the River Meuse, Netherlands (Corkum et al. 2004; Van Kessel et al. 2016). In Yunnan Province, *Rhinogobius cliffordpopei* has become the dominant benthic fish in Erhai Lake. Competition for food resources with native species (e.g., *Barbodes daliensis*, *Cyprinus barbatus* and *Cyprinus longipectoralis*) is one of the major causes of population declines of native species. *Rhinogobius cliffordpopei* mainly preys on zooplankton (cladocerans and copepods) and aquatic insects (chironomid larvae). In addition to competing with native fish for food resources, *Rhinogobius cliffordpopei* often prey on fish larvae and eggs, including those from native species (Du and Li 2001; Xie et al. 2000), which could also adversely affect native fish.

Of particular concern are 24 non-native fish species with increased risks of potential invasion (i.e., BRA+CCA scores) under future climate conditions, accounting for 64.86% of screened species. This suggests that more than half of the non-native fish species will pose higher threats to native fish in the future. A study by Liu et al. (2019) also suggested that biological invasions would pose a higher threat to freshwater fish in southwest China in the future. Notably, BRA+CCA scores of two fish species decreased under future climate conditions, namely *Neosalanx taihuensis* and *Salmo trutta*. *Salmo trutta* even decreased from high risk to medium risk. This result was also found in a risk screening of potential invasiveness in the West Siberian Plain, Russia, where salmonids would be at reduced relative risk under future climate changes (Interesova et al. 2020). The reduced risk of *Neosalanx taihuensis* invasion is related to the local invasion control of this species. Targeted fishing can effectively control the spread and population development of *Neosalanx taihuensis*. However, *Neosalanx taihuensis*, the most invasive non-native fish in Yunnan plateau lakes, is still at high risk of invasion in the future.

Overall, the AS-ISK decision-support tool was used successfully to identify non-native fishes with a high risk of invasion in Yunnan Province. These results will provide a basis for policymakers and managers to address the problems of invasive fishes in the region. Four of the 20 non-native fishes listed as high risk were ranked as posing a “very high risk” of being invasive (i.e., *Hypostomus plecostomus*, *Oreochromis niloticus*, *Oreochromis mossambicus* and *Oreochromis aureus*) and therefore represent the highest priority species for management and control. The severe and complex problems of non-native fish invasion in Yunnan Province, coupled with lagging research, have resulted in little or no documented data on the adverse effects of non-native fishes (e.g., ecological and socio-economic effects and effects on ecosystem services). In particular, population dynamics, especially population size and reproductive status, of high-risk non-native fish should be studied in the future. Much of the information used in the present study is based on how species behaved in other invaded areas. Such knowledge gaps regarding high-risk fish species highlight areas for future research on non-native fishes in Yunnan Province to guide future policy and management decisions. Furthermore, with the deepening of the research and understanding on non-native species, relevant policies and laws have been formulated for management of non-native species in China. In particular, the "Measures for the Management of Invasive Alien Species" promulgated in 2022 clearly states that targeted fishing and other measures should be taken against non-native aquatic animals. AS-ISK can accurately identify the invasive risk of alien species and provide management priority, which is in line with China's current demand for non-native species management.

Authors' contribution

All authors contributed to the study conception and design. You Ge: Conceptualization, Methodology, Software, Writing – original draft, Writing – review and editing. Xiaohong Gu: Conceptualization, Data curation, Supervision, Writing – review and editing. Qingfei Zeng: Conceptualization, Data curation, Methodology, Supervision. Zhigang Mao: Investigation, Data curation, Methodology, Supervision. Huihui Chen: Methodology. Huiting Yang: Methodology, Supervision.

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Supplementary material

The following supplementary material is available for this article:

Table S1. *Acheilognathus chankaensis*
Table S2. *Acheilognathus macropterus*
Table S3. *Acipenser baerii*
Table S4. *Ameiurus nebulosus*
Table S5. *Anguilla anguilla*
Table S6. *Anguilla japonica*
Table S7. *Carassius cuvieri*
Table S8. *Clarias gariepinus*
Table S9. *Ctenopharyngodon idella*
Table S10. *Cultrichthys erythropterus*
Table S11. *Gambusia affinis*
Table S12. *Hypomesus olidus*
Table S13. *Hypophthalmichthys molitrix*
Table S14. *Hypophthalmichthys nobilis*
Table S15. *Hyporhamphus intermedius*
Table S16. *Hypostomus plecostomus*
Table S17. *Ictalurus punctatus*
Table S18. *Labeo rohita*
Table S19. *Megalobrama amblycephala*
Table S20. *Micropterus salmoides*
Table S21. *Mylopharyngodon piceus*
Table S22. *Neosalanx taihuensis*
Table S23. *Oncorhynchus mykiss*
Table S24. *Oreochromis aureus*
Table S25. *Oreochromis mossambicus*
Table S26. *Oreochromis niloticus*
Table S27. *Parabramis pekinensis*
Table S28. *Paramisgurnus dabryanus*
Table S29. *Piaractus brachypomus*
Table S30. *Prochilodus lineatus*
Table S31. *Protosalanx hyalocranius*
Table S32. *Rhinogobius cliffordpopei*
Table S33. *Salmo trutta*
Table S34. *Sarcocheilichthys nigripinnis*
Table S35. *Siniperca chuatsi*
Table S36. *Tinca tinca*
Table S37. *Toxabramis swinhonis*

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