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RESEARCH ARTICLE

Invasiveness screening of non-native fishes for the middle reach of the Yarlung Zangbo River, Tibetan Plateau, China

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Abstract

The aim of this study was to identify potentially invasive non-native freshwater fishes in the middle reach of the Yarlung Zangbo River, Tibetan Plateau (China), using the Aquatic Species Invasiveness Screening Kit (AS-ISK), as decision-support tool. Based on independent evaluations of 24 non-native freshwater fishes, receiver operating curve analysis identified a threshold score of ≥ 29 for distinguishing species likely to pose a high risk of becoming invasive from species likely to pose low-to-medium risk (< 29) in the risk assessment area. Nine species were categorized as "high risk": goldfish *Carassius auratus*, topmouth gudgeon *Pseudorasbora parva*, brook trout *Salvelinus fontinalis*, Oriental weatherfish (a.k.a. dojo gudgeon) *Misgurnus anguillicaudatus*, Siberian taimen *Hucho taimen*, common carp *Cyprinus carpio*, peled *Coregonus peled*, western mosquitofish *Gambusia affinis*, and Chinese rice fish *Oryzias sinensis*. The three lowest scoring species were Arctic cisco *Coregonus autumnalis*, Wuchang bream *Megalobrama amblycephala*, and Chinese ice fish *Neosalanx taihuensis*, which are unlikely to be invasive because they are unable to complete their life cycle in the risk assessment area. Climate change assessments scores increased or remained the same for warm-water species and decreased for coldwater species. This study was the first application of AS-ISK in western China, and the results suggest that AS-ISK is a useful and valid tool for identifying potentially invasive risk aquatic species in China.

KEYWORDS

alien species, AS-ISK, large rivers, risk identification, Tibetan Plateau

1 | INTRODUCTION

China is extremely diverse in terms of fish fauna, with 1,363 species of native freshwater fish (Zhang & Zhao, 2016). However, increased human activities such as aquarium, aquaculture, and fisheries trades have brought non-native (NN) freshwater fish species into China not only from other countries but also from different drainage basins within the same country (Gozlan, Britton, Cowx, & Copp, 2010). Recent studies have revealed that China possesses the largest number of NN freshwater fishes of any country, encompassing 439 species belonging to 22 taxonomic orders, 67 families, and 256 genera, of these, 53 species (12.1%) have established self-sustained populations (Xiong, Sui, Liang, & Chen, 2015).

The Tibetan Plateau, regarded as "the engine of the global climate system," is significantly affected by introductions of NN freshwater fishes, with species introduced to the Yarlung Zangbo River and its

tributaries either intentionally or accidentally. There have been few reports of the presence and absence of NN fishes for the Yarlung Zangbo River. And despite the need to identify the potential threats they pose, no risk analysis have been undertaken of these species (Sui, Zhang, Jia, Chen, & He, 2016; Yang et al., 2010). The middle reach of the Yarlung Zangbo River extends 1,293 km, including a few major tributaries and their flood plains, such as the Lhasa and Niyang rivers (Encyclopedia of Rivers and Lakes in China, 2014). The Yarlung Zangbo's middle reach also represents the agricultural, industrial, political, and cultural centre of Tibet, with several large cities (Lhasa, Shigatse, Shannan, and Nyingchi) and over half of the Tibet population. Previous research indicated biological invasions often occurred as a result of human activities, and the Yarlung Zangbo ecosystem is very sensitive and vulnerable. Once NN species successfully invaded, they are difficult to be eradicated and may cause irreversible negative effects (Gozlan et al., 2010), so the aim of this study was to identify

NN fishes that are likely to pose a high risk of becoming invasive in the middle reach of the Yarlung Zangbo River, that is, the risk assessment (RA) area. The results of this risk screening process are intended to inform policy and management of NN fishes in the middle reach of this important river system.

2 | MATERIALS AND METHODS

2.1 | Study area

The middle reach of the Yarlung Zangbo River (the RA area), which has a length of 1,293 km, starts from the village of Lizi, Zhongba County, and ends at Paizhen in Milin County (Figure 1). Air temperatures in the RA area during 1961–2010 varied 0.1–14.5 °C, and this has been predicted to increase by 2.2–2.6 °C in the next 50 years (Gesang, Nimaji, Ma, & Hong, 2013; Nan, Li, & Cheng, 2005). The future status of each species was predicted based on water temperature (T_w) calculated from air temperature (T_a) by $T_w = 3.47 + 0.898T_a$ (Erickson & Stefan, 1996; Nan et al., 2005). Therefore, water temperature of the middle reach of the Yarlung Zangbo River will be 5.5–18.8 °C in 50 years. Annual precipitation is 946 mm, with 60% to 90% falling between June and September. Non-native species have been introduced to the RA area either by non-Tibetans as a food supply or by Tibetans as traditional Buddhist practices (Sui et al., 2016; Zhou et al., 2013). Owing to the vulnerable ecological conditions and unique ichthyologic fauna, some of the NN species may already successfully invaded into our RA area.

2.2 | Risk analysis

To identify potentially invasive species with respect to the RA area, we used the Aquatic Species Invasiveness Screening Kit (AS-ISK), which is

available for free download (www.cefas.co.uk/nns/tools/) and described in detail elsewhere (Copp et al., 2016). Intended as an enhanced replacement for the well-established risk identification tool, the Fish Invasiveness Screening Kit (FISK; reviewed in Copp, 2013), AS-ISK is compliant with the “minimum requirements” (Roy et al., 2015) for the assessment of NN species for the EC Regulation on the prevention and management of the introduction and spread of invasive alien species (European Commission, 2014). As with FISK, AS-ISK consists of 49 questions on life-history traits, biology, ecology, biogeography, and history of introduction, and it is also complemented by additional six Climate Change Assessment (CCA) questions to predict how future climate conditions are likely to affect the AS-ISK assessment with respect to risks of introduction, establishment, dispersal, and impact (Glamuzina et al., 2017; Tarkan, Sari, İlhan, Kurtul, & Vilizzi, 2016; Tarkan et al., 2017).

For the RA area, 24 NN freshwater fish species were selected for AS-ISK assessment based on the four criteria intended to assess potential future NN species within the context of existing NN species (Tarkan et al., 2017): (a) those translocated (i.e., native to other parts of China but not to the RA area) have not established self-sustaining populations in the RA area (four species); (b) those already introduced to the RA area and have established self-sustaining populations (six species); (c) those not yet present in the RA area but already invaded in the adjacent area (six species); and (d) those not yet present in the RA area but important for aquarium and aquaculture trade (eight species). Both intentionally and accidentally introduced species were included in our criteria. Two experts (D. He and R. Yang) experienced in fishes of the Yurlung Zangbo River carried out all of the AS-ISK assessments together as a single assessment.

Similar to Gordon, Onderdonk, Fox, and Stocker (2008) and various FISK applications (reviewed in Copp, 2013), calibration of the AS-ISK medium-to-high risk threshold for the RA area was undertaken by receiver operating characteristic (ROC) analysis (Bewick, Cheek, & Ball, 2004) to assess the predictive ability of AS-ISK, whereby the eventual purpose is to determine a threshold (or “cut-off value”) to discriminate between potentially invasive and non-invasive species (Glamuzina et al., 2017; Tarkan et al., 2016, 2017). As per Copp et al. (2009) and subsequent applications of risk screening toolkits of the “ISK” type (reviewed by Copp, 2013), each species was assigned a priori classification as either “invasive” or “non-invasive” with respect to their perceived threat to humans (invasive/non-invasive) and to their IUCN Red List Status (not evaluated/least concern/vulnerable/near threatened) based on information in Fishbase (www.fishbase.org). The sensitivity of the AS-ISK test will be the proportion of a priori invasive fish species that are correctly identified as invasive by the test, whereas specificity refers to the proportion of a priori non-invasive fish species that are correctly identified as such. The area under the ROC curve is a key measure of the accuracy of the calibration analysis: with an area = 1.0, the ROC curve consists of two straight lines, one vertical line from 0.0 to 0.1 and the other, horizontal line, from 0.1 to 1.1; this indicates that the test is 100% accurate because both the sensitivity and specificity are 1.0. In other words, there are no false positives and no false negatives. Whereas, a ROC curve that is the diagonal line from 0.0 to 1.1 indicates the test cannot discriminate—the ROC area for this line is 0.5. Typically, ROC

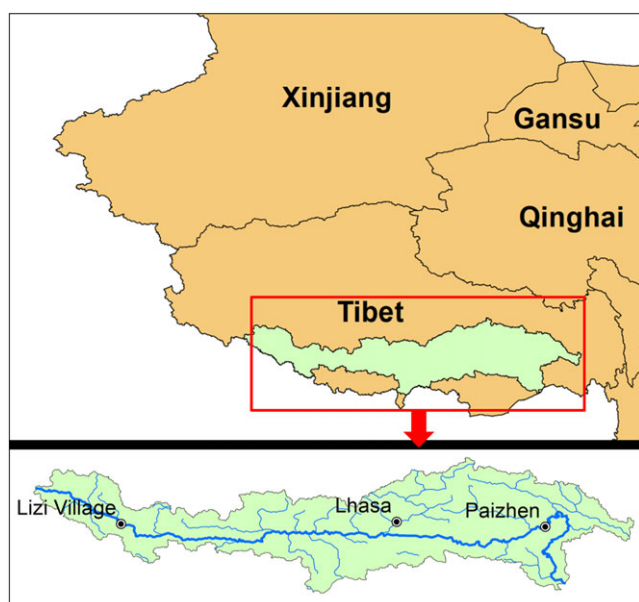


FIGURE 1 Map of the Yarlung Zangbo River within China. Middle reach of the Yarlung Zangbo River is from the village of Lizi in Zhongba County to Paizhen in Milin County [Colour figure can be viewed at wileyonlinelibrary.com]

curve areas range from 0.5 to 1.0. ROC analysis was taken using “pROC” in the R (version 3.3.2, Lucent Technologies) of which the bootstrap replicates were 2,000× (Xavier et al., 2011).

Youden-J statistic was used to calculate the threshold score for the RA area. The score that maximized the opportunity of the invasive species being judged as invasive and minimized the opportunity of the invasive species being judged as non-invasive was chosen as the threshold. Therefore, when a species received an invasiveness score that is larger than or equal to the threshold, that species was classed as invasive; if not, then the species was classed as non-invasive. Student's *t*-test was used to determine if differences ($\alpha = .05$) existed between scores for preassigned “invasive” and “non-invasive” groups. A confidence factor was calculated for the assessment of each species as

$$\sum(CQ_i)/(4 \times 55) (i = 1, 2, \dots, 55)$$

where CQ_i is the confidence level for each assessment question (1 = low; 2 = medium; 3 = high; and 4 = very high), and the value should range from 0.25 (all questions receive low confidence level) to 1 (all questions receive very high confidence level).

3 | RESULTS

For the list of assessed species, the areas under the ROC curves were always significantly >0.5, which indicates that AS-ISK was able to distinguish accurately between invasive and non-invasive species to a degree greater than would be expected by chance alone. The maximum Youden's index value (0.25) for the RA area corresponded to a threshold score of 29. Therefore, among the 24 assessed species, nine (interval [29, 37]) were classed as being of high invasiveness risk, and 15 (interval [9.5, 29]) of low invasiveness risk (Table 1). The area under the smoothed ROC curve was 0.71 (0.50–0.81, 95% CI; Figure 2). This number fits in the range of (0.5, 1), which indicates that AS-ISK was able to recognize species of high risk from those of medium-to-low risk of becoming invasive in the RA area.

The three highest-scoring species were goldfish *Carassius auratus*, topmouth gudgeon *Pseudorasbora parva*, and brook trout *Salvelinus fontinalis*, followed by the other six species posing moderately high invasiveness risk: Oriental weatherfish (a.k.a. dojo gudgeon) *Misgurnus anguillicaudatus*, Siberian taimen *Hucho taimen*, common carp *Cyprinus carpio*, peled *Coregonus peled*, western mosquitofish *Gambusia affinis*, and Chinese rice fish *Oryzias sinensis*. The three species with the lowest invasiveness scores were Arctic cisco *Coregonus autumnalis*,

TABLE 1 Invasiveness results of the 24 non-native freshwater fish species in the middle reach of the Yarlung Zangbo River assessed with Aquatic Species Invasiveness Screening Kit (BRA = Basic Risk Assessment; CCA = Climate Change Assessment)

Criteria	Scientific name	Common name	BRA score	CCA score	Invasiveness	Outcome	Confidence factor
3	<i>Coregonus autumnalis</i>	Arctic cisco	9.5	9.5	0	Non-invasive	0.83
4	<i>Megalobrama amblycephala</i>	Wuchang bream	13.5	13.5	0	Non-invasive	0.75
4	<i>Neosalanx taihuensis</i>	Chinese ice fish	15.0	23.0	0	Non-invasive	0.70
3	<i>Hemiculter leucisculus</i>	Sharpbelly	17.5	25.5	1	Non-invasive	0.83
2	<i>Culter alburnus</i>	QiaoZuiBo	19.5	27.5	1	Non-invasive	0.88
1	<i>Micropercops swinhonis</i>	XiaoHuangYouYu	20.0	20.0	1	Non-invasive	0.80
3	<i>Rhinogobius giurinus</i>	ZiLingWenXiaHu	21.5	27.5	0	Non-invasive	0.91
3	<i>Rhinogobius cliffordpopei</i>	BoShiWenXiaHu	21.5	27.5	0	Non-invasive	0.91
2	<i>Silurus asotus</i>	Amur catfish	22.0	28.0	0	Non-invasive	0.85
2	<i>Abbottina rivularis</i>	Chinese false gudgeon	24.0	24.0	0	Non-invasive	0.82
1	<i>Hypophthalmichthys molitrix</i>	Silver carp	27.0	31.0	1	Non-invasive	0.85
3	<i>Aristichthys nobilis</i>	Bighead carp	27.0	31.0	0	Non-invasive	0.65
4	<i>Coregonus muksun</i>	Muksun	28.0	22.0	0	Non-invasive	0.75
1	<i>Ctenopharyngodon idellus</i>	Grass carp	28.0	34.0	1	Non-invasive	0.85
1	<i>Paramisgurnus dabryanus</i>	DaLingFuNiQiu	28.0	36.0	0	Non-invasive	0.80
4	<i>Oryzias sinensis</i>	Chinese rice fish	29.0	31.0	0	Invasive	0.68
3	<i>Gambusia affinis</i>	Western mosquitofish	29.0	31.0	1	Invasive	0.70
4	<i>Coregonus peled</i>	Peled	30.0	24.0	1	Invasive	0.73
2	<i>Cyprinus carpio</i>	Common carp	30.0	40.0	1	Invasive	0.89
4	<i>Hucho taimen</i>	Siberian taimen	31.0	29.0	0	Invasive	0.79
2	<i>Misgurnus anguillicaudatus</i>	Oriental weatherfish	33.0	43.0	1	Invasive	0.82
4	<i>Salvelinus fontinalis</i>	Brook trout	35.0	37.0	1	Invasive	0.87
4	<i>Pseudorasbora parva</i>	Topmouth gudgeon	35.0	39.0	1	Invasive	0.85
2	<i>Carassius auratus</i>	Goldfish	37.0	45.0	1	Invasive	0.88

Note. In the category of criteria: 1 = non-native species already present in Tibet Plateau; 2 = non-native species already establish self-sustaining populations in Tibet Plateau; 3 = non-native species not yet present in Tibet Plateau but already invaded in the adjacent area; 4 = non-native species not yet present in Tibet Plateau but already invaded in the adjacent area. Species without English common name is given an alphabetic Chinese name. For each species, the precategorized invasiveness is based on the information on Fishbase (www.fishbase.org): 0 = non-invasive; 1 = invasive.

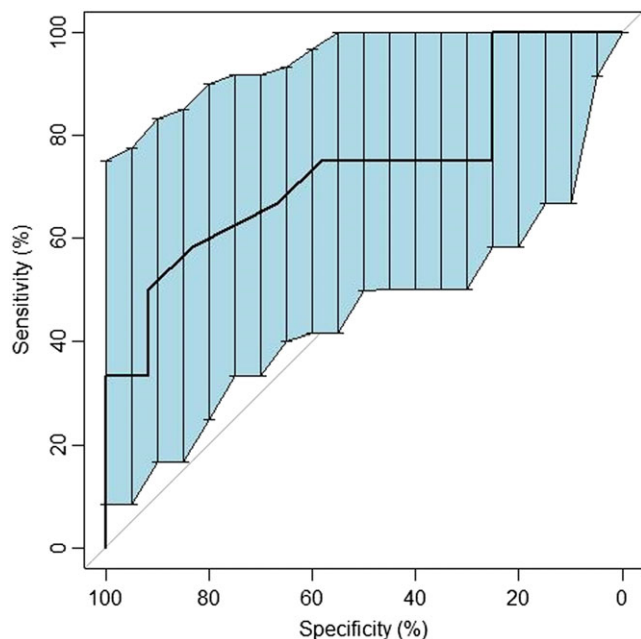


FIGURE 2 Receiver operating characteristic curve of Aquatic Species Invasiveness Screening Kit scores for 24 non-native freshwater fish species in the middle reach of the Yarlung Zangbo River. Note. Sensitivity (%) = the probability of the invasive species being correctly identified as invasive; specificity (%) = the probability of the non-invasive species being correctly identified as non-invasive. Area under the curve = accuracy of the analysis, 1 = 100% accurate; 0.5 = 0% accurate [Colour figure can be viewed at wileyonlinelibrary.com]

Wuchang bream *Megalobrama amblycephala*, and Chinese ice fish *Neosalanx taihuensis*. Twelve species were precategorized as “invasive” species and the other half were precategorized as “non-invasive” species. Mean value of the “invasive” group was 28.4, which was significantly different than 22.5 for the “non-invasive” group ($p = .0395$).

Increased invasiveness risk under climate change condition for 17 species was revealed by the CCA scores, with decreased invasive risk for three species, and no change for four species (Table 1). The three species that received lower CCA scores are all coldwater, temperate zone species (Siberian taimen, peled, and muksun *Coregonus muksun*). The species with higher CCA scores are mostly warm-water, subtropical species with wide distributional range, where goldfish, Oriental weatherfish, and common carp received the largest increase from Basic Risk Assessment (BRA) to CCA scores. The four species with no change in BRA scores to CCA scores due to predicted climate conditions were XiaoHuangYouYu *Micropercops swinhonis*, Chinese false gudgeon *Abbottina rivularis*, Wuchang bream, and Arctic cisco.

Mean confidence for the assessments was 3.23 ± 0.06 SE, and mean confidence factor was 0.81 ± 0.02 SE. ZiLingWenXiaHu *Rhinogobius giurinus* received the highest confidence of 3.65 ± 0.08 SE, and bighead carp *Aristichthys nobilis* received the lowest confidence of 2.62 ± 0.08 SE (Table 1).

4 | DISCUSSION

The AS-ISK assessments of NN fishes for the Yarlung Zangbo River revealed BRA and CCA threshold scores of 29 and 31, which are

similar to those (both 28) recently calibrated for Turkey (Tarkan et al., 2017) but higher than those (10.0 and 12.6, respectively) for the trans-Balkans River Neretva (Glamuzina et al., 2017) and the very low threshold score (-3.7) reported for Lake Marmara, West Anatolia, Turkey (Tarkan et al., 2016). Differences in threshold values in FISK screening kits are well documented (review in Copp, 2013), being attributed to the number of translocated species (Glamuzina et al., 2017; Simonović et al., 2013). Other possible influences are hydrological characteristics and severe climate conditions, which could explain why only eight of the many introduced NN species have established self-sustaining populations in this RA area (Sui et al., 2016).

Most of the NN fishes with high AS-ISK scores were species used in aquaculture or those with long introduction histories in China. For example, goldfish introductions began hundreds of years ago for human consumption and for ornamental purposes (Welcomme, 1988), and these introductions were facilitated by the species' tolerance of temperature fluctuations and low dissolved oxygen concentrations (Spotila, Terpin, Koons, & Bonati, 1979; Walker & Johansen, 1977). The successful invasion of goldfish throughout the world may suggest its higher risk of being invasive in the middle reach of the Yarlung Zangbo River. Brook trout, Siberian taimen, and peled are all common species in coldwater aquaculture. They are native to the Great Lakes of North America and/or the Arctic Ocean basin, which have similar climate types to the present RA area (Peel, Finlayson, & McMahon, 2007). Although these coldwater species have not yet been reported for the Yarlung Zangbo River, the AS-ISK scores (Table 1) indicate that these species would be likely to establish self-sustaining populations if introduced to the RA area. Common carp has also been widely introduced around the world (Weber & Brown, 2009), with adverse consequences for the recipient ecosystems (Vilizzi, Ekmekçi, Tarkan, & Jackson, 2015). It was introduced to the Yarlung Zangbo River as a preferred food supply of the Han People (Sui et al., 2016). Small-sized species such as topmouth gudgeon, Chinese rice fish, and western mosquitofish were accidentally introduced to the Yarlung Zangbo River. They all possess a demonstrated ability to adapt to novel ecosystems, and their small body sizes likely to facilitate their survival and establishment in the Yarlung Zangbo River. Also introduced as food supply was the Oriental weatherfish, which has established breeding populations in the shallow rivers and swamps in the vicinity of cities and towns. In general, species with higher AS-ISK scores were mostly highly adaptable and tolerant coldwater or cool water species with long introduction history.

Species that attracted a score <29 pose a reduced (low-to-medium) likelihood of becoming invasive in the RA area, though some do occur in the middle reach of the Yarlung Zangbo River. Our results suggested that they might have difficulties in establishing populations due to one or more critical criteria. For example, silver carp *Hypophthalmichthys molitrix* and bighead carp were introduced for aquaculture purposes, but they require a temperature of $18\text{--}20^\circ\text{C}$ for reproduction, and therefore, they are unlikely to reproduce successfully in the climatic conditions of RA area, where 16.6°C is the highest temperature recorded in the past 50 years (Gesang et al., 2013). Arctic cisco, which migrates out to sea as young juveniles to grow on and mature before returning up river and spawn (Svetovidov, 1984), received the lowest

AS-ISK score. Chinese ice fish were mainly introduced to the lakes on the Yunnan-Kweichow Plateau, which is to the east of Tibetan Plateau and achieved success with an area of 430 km² (Zhou & Huang, 2005). However, there is no record of intentional introduction of Chinese ice fish into Yarlung Zangbo River, and this species is more likely to establish self-sustaining populations in lakes and reservoirs than in rivers (Kang, Deng, Wang, & Zhang, 2013; Liu, Li, & Xiong, 2016), therefore, this species received a low AS-ISK score. This study suggests that Chinese false gudgeon and Amur catfish *Silurus asotus* are unlikely to be invasive, but juveniles of these two species have been discovered in the Yarlung Zangbo River. Their low AS-ISK scores may be due to the absence of published evidence that demonstrates adverse ecological consequences following their introduction. Correspondingly, they received lower scores on the indexes of biology/ecology (Sui et al., 2016). Adult DaLingFuNiQiu *Paramisgurnus dabryanus* and grass carp *Ctenopharyngodon idellus* have also been found in the Yarlung Zangbo River, where aquatic plants are rare and natural reproduction of these fishes was not observed during our study nor reported in the literature. Muksun have been found in Qinghai, Northern part of Tibetan Plateau but not the Yarlung Zangbo River (Tang & He, 2015), and there have been no negative impacts reported following XiaoHuangYouYu introductions. Little biological or ecological information is available for QiaoZuiBo *Culter alburnus*; however, the species' wide natural distribution (from Mongolia to Hainan) suggests a broad environmental adaptability (www.fishbase.org).

Eleven species received very high CCA scores, suggesting they are likely to benefit from climate warming: silver carp, bighead carp, Chinese rice fish, western mosquitofish, grass carp, DaLingFuNiQiu, brook trout, topmouth gudgeon, common carp, Oriental weatherfish, and goldfish. The AS-ISK scores for peled and Siberian taimen decreased from "invasive" based on BRA scores to "non-invasive" under conditions of climate warming, which is consistent with their coldwater status. For example, peled and muksun spawn at 0–2.0 °C (www.fishbase.org), so they may not be able to spawn in the RA area when the lowest water temperature will be 5.5 °C. Increased risk ranking (from BRA to CCA) for Chinese rice fish, bighead carp, grass carp, and silver carp is probably due to their higher risk of entry, establishment, and dispersal in the RA area under future climate conditions. For example, goldfish spawns and grows well at temperatures >15 °C, and silver carp and bighead carp both require a temperature of 18–20 °C for reproduction. Whereas the water range for Oriental weatherfish ranges 5–25 °C (www.fishbase.org; GISD). Therefore, the projected water temperature in 50 years (5.5–18.8 °C) is likely to facilitate their establishment and dispersal in the RA area.


Of the three initial calibrations of AS-ISK, the present is the first application for fishes in an RA area, which encompasses both the more extreme "cold" and "arid" plateau climate zones (Peel et al., 2007). However, some FISK applications, such as in Mexico (Mendoza, Luna, & Aguilera, 2015), have included the "arid" climate zone. In summary, AS-ISK provided a good way to identify potentially invasive NN species across a broad range of environments, suggesting that some NN fishes could encounter difficulty in establishing and spreading in the middle Yarlung Zangbo River due to its remote location and severe climate conditions. However, modern means of transportation, increasing economic trade and certain religious practices, may combine

to facilitate biological translocations, leading to localized invasions, in the Yarlung Zangbo River (Gozlan et al., 2010). To avoid adverse ecological and economic consequences, risk analysis should include pathway analysis (Copp et al., 2016) to identify potential introduction vectors (e.g., Copp, Templeton, & Gozlan, 2007) such as aquaculture and aquarium/pet trade (Copp et al., 2016), fish movements (e.g., Copp, Vilizzi, & Gozlan, 2010a), and other demographic-related pathways (e.g., Copp, Vilizzi, & Gozlan, 2010b). In view of the variation in threshold score values already observed in the initial three applications of AS-ISK (Glamuzina et al., 2017; Tarkan et al., 2016, 2017), invasiveness screening tools such as AS-ISK should be calibrated for each RA area where applied (Copp, 2013), whether that be elsewhere in China or elsewhere worldwide. This does not, however, negate the potential utility of meta-analyses to assess global-scale patterns in risk thresholds (e.g., Gordon et al., 2008).

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